GENOMIC ANALYSIS OF DOMESTICATION-RELATED TRAITS AND STEM RUST RESISTANCE IN TETRAPLOID WHEAT

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ABSTRACT

Modern durum and common wheat cultivars were developed from ancient wheat ancestors by natural and artificial selection of agronomic and domestication traits, which ultimatey decreased their genetic diversity and made them more susceptible to various biotic and abiotic stresses. At present, new sources of resistance need to be introgressed into future wheat cultivars to combat the effect of the disease stem rust caused by the biotrophic fungal pathogen *Puccinia graminis* f.sp. *tritici* (*Pgt*). In this dissertation, I first analyzed the domestication-related traits in a tetraploid recombinant inbred line (RIL) population developed from a cross between the durum wheat line Rusty and the cultivated emmer accession PI 193883 (referred to as the RP883 population). Second, the RP883 population and a double haploid (DH) population (referred to as the LP749 population) derived from a cross between the durum cultivar Lebsock and the *Triticum*. *turgidium* ssp. *carthlicum* accession PI 94749, and nine durum wheat cultivars were screened with *Pgt* races TMLKC, TTKSK, TRTTF, and TTTTF. Domestication-related trait analysis in the RP883 population showed vernalization (*Vrn-A1*) and domestication (*Q*) genes had a pleiotrophic effect on spike length, spikelet per spike, spike compactness, and threshability. Additionally, an interaction and dosage effect of three free-threshing trait governing loci, teneacious glume Tg^{2A} and Tg^{2B}, and *q*, revealed that mutation in all three loci are required to attain complete free threshability. The stem rust analysis done in the RP883 population showed two *Sr* gene regions conferring resistance against TMLKC, TTKSK, and TRTTF: one novel gene region on chromosome 2BL (*Sr883*) and likely a new allele or gene residing in close proximity to the *Sr13* gene on 6AL. The second stem rust study using the LP749 population and nine durum wheat cultivars showed that most likely the U.S. durum germplasm carries the four major *Sr* genes, *Sr7a* (4AL), *Sr8155B1* (6AS), *Sr13* (6AL), and likely

Sr9e (2BL) against TTKSK, TRTTF, and TTTTF. In conclusion, results obtained from this domestication study provide knowledge about different stages in wheat evolution. Both stem rust studies revealed genetic diversity in the tetraploid wheat gene pool and indicate their utility in future breeding programs.

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DEDICATION

To my beloved parents

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1. INTRODUCTION

Wheat is the third most important food crop after maize and rice and provides approximately 20% of the calories in the daily human diet. Among the different forms of wheat, hexaploid common (bread) wheat (*Triticum aestivum* ssp *aestivum* L., 2*n* = 6*x* = 42, AABBDD) and tetraploid durum wheat (*T. turgidium* ssp. *durum* (Desf.) Husnot, 2*n* = 4*x* = 28, AABB) are the two main types cultivated and consumed around the world. Modern durum wheat is the result of the evolution of wild emmer wheat [*T. turgidium* ssp. *dicoccoides* (Körn) Thell, 2*n* = 4*x* = 28, AABB], which came into existence through an amphiploidization event between two diploid species: *T. urartu* Tumanian ex Gandylian (2*n* = 2*x* =14, AA) (Dvorak et al. 1993) and a close relative of *Aegilops speltoides* ssp. *lingustica* (SS) (Faris 2014 for review).

About 10,000 years ago, brittle rachis-containing wild emmer wheat further evolved into the tough rachis cultivated emmer wheat (*T. turgidium* ssp. *dicoccum* L., 2*n* = 4*x* = 28, AABB), which has a nonbrittle rachis (Faris 2014 for review). Although cultivated emmer had non-brittle rachis, it still had hulled seeds and was thus not free threshing. This non-free threshing cultivated emmer transitioned into tetraploid durum wheat by acquiring the free-threshing trait (Faris 2014 for review). In addition to free-threshing and non-shattering traits, current durum wheat varieties also carry other improved agronomic characteristics such as shorter plant height, shorter spike length, increased seed size, etc. In the process of domestication, the domesticated wheat varieties have become less genetically diverse compared to its wild ancestors. This loss of diversity makes modern wheat varieties more prone to various biotic and abiotic stresses. Every year, 30-60% of the yield is lost due to biotic and abiotic stresses (Varshney et al. 2014).

Since ancient times wheat production has been under continuous threat from various fungal, bacterial, viral, and nematode pathogens, and insect pests. Among these, wheat stem rust,

or black rust, caused by a fungal pathogen *Puccinia graminis* f.sp. *tritici* Eriks & E.Henn (*Pgt*) is currently considered a major threat to food security. During 1998, the most virulent *Pgt* race, TTKSK (a member of the Ug99 group), was detected in Uganda, and found to be virulent even on the widely deployed stem rust resistance gene *Sr31* (Singh et al. 2008; Pretorius et al. 2000). To date, 13 variants among the Ug99 group have been detected in 13 different countries (http://Rusttracker.cimmyt.org; Fetch et al. 2016). The high mobility, and mutation and recombination rate of this pathogen pose a serious threat to wheat production throughout the world (Jin 2005; Singh et al. 2008; De wolf et al. 2011; Hulbert and Pumphrey 2014; Lopez-Vera et al. 2014; http://Rusttracker.cimmyt.org). At present, more than 80% of the wheat-producing area is threatened by the Ug99 group of races (Lopez-Vera et al. 2014). Compounding this dilemma is the projection that by 2050, the world population will reach nearly 9 billion (Ronald 2014). To feed such a large population, agricultural outputs need to be increased by developing new wheat varieties with high yield potential and resistance against diseases and pests (Tester and Langridge 2010; Ronald 2014; Varshney et al. 2014; Hulbert and Pumphrey 2014).

Development of rust-resistant varieties is a major objective of breeding programs to effectively control the disease (Hulbert and Pumphrey 2014). Numerous genomic tools are now available that allow rapid mapping and marker development for novel genes, greatly expediting the possible deployment of new resistance genes. Having knowledge of different stages of wheat evolution and the wheat domestication process will also be beneficial for taking advantage of appropriate wheat accessions for future breeding strategies.

In this dissertation, I first describe the characterization of domestication-related traits that emerged during the evolution from cultivated emmer to durum wheat in a tetraploid recombinant

inbred line (RIL) population. Secondly, I conducted mapping of stem rust resistance genes derived from durum and cultivated emmer wheat using tetraploid doubled haploid and RIL populations.

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2. LITERATURE REVIEW

2.1. Wheat evolution

Both progenitor genera of wheat, *Triticum* and *Aegilops*, arose around 3.0 million years ago (MYA) from a common ancestor having 7-chromosomes (Faris 2014 for review). The amphiploidization took place between *T. urartu* Tumanian ex Gandylian ($2n = 2x = 14$, AA) (Dvorak et al. 1993) and a close relative of *Ae. speltoides* ssp. *lingustica* (SS) around 0.5 MYA (Faris 2014 for review). This event led to the formation of wild emmer *T. turgidium* ssp. *dicoccoides* (Körn.) Thell ($2n = 4x = 28$, AABB), which evolved into cultivated emmer [*T. turgidium* ssp. *dicoccum* (Schrank) Schübl, $2n = 4x = 28$, AABB] having a non-brittle rachis (*br*) (Faris 2014 for review). Wild emmer habitat is confined to the Fertile Crescent region (from the southern Levant across Israel and Lebanon to southeastern Turkey and across northern Iraq and northwestern Iran) (Faris 2014 for review). The Karacadag region was the most likely site for the origin of cultivated emmer (Faris 2014 for review). Cultivated emmer evolved to give rise to free-threshing durum wheat (*T. turgidium* ssp. *durum* L., $2n = 4x = 28$, AABB*)* by the transition of primitive tenacious glumes (*Tg*) and non-free threshing (*q*) genes to the domesticated non-tenacious glumes (*tg*) and free threshing characteristics (*Q*) (Faris 2014 for review).

Of these three major domestication genes, *Q* and *br* have been cloned and are located on chromosome 5A and chromosomes 3A and 3B, respectively (Faris et al. 2003; Simons et al. 2006; Avni et al. 2017). The *Q* gene belongs to the *APETALA2* (*AP2*) family of transcription factors. It is also known as the super domestication gene due to its pleiotropic effect on multiple agronomic traits such as plant height, spike length, spikelet per spike, threshability, rachis fragility, etc (Faris and Gill 2002; Faris et al. 2005; Simons et al. 2006; Faris 2014 for review). The significant trait changes caused by the transition of *q* to *Q* redefined modern agriculture

(Muramatsu 1986; Simons et al. 2006; Zhang et al. 2011; Faris et al. 2014 b). The *Q* gene differs from the primitive allele *q* in that is has enhanced homodimer formation (due to $Valine_{329}$ to Isoleucine mutation) and increased levels of transcription (Simons et al. 2006). Recent studies conducted by Greenwood et al. (2017) and Debernardi et al. (2017) showed that the increased transcription level is related to a single point mutation in the microRNA172 binding site of *q* gene (at exon 9), which regulates the expression and function of *Q*.

The second free threshing governing gene, *tg*, is located on homoeologous group 2 in the wheat genome (Kerber and Rowland 1974; Simonetti et al. 1999; Jantasuriyarat et al. 2004; Nalam et al. 2007; Sood et al. 2009; Dvorak et al. 2012; Faris et al. 2014a, b). The presence of *Tg* is known to prevent the free threshing of wheat even in the presence of *Q*, suggesting that *Tg* has an epistatic effect on *Q* gene function (reviewed in Faris 2014). This gene was first mapped on chromosome arm 2DS in hexaploid wheat (Kerber and Rowland 1974). Later on, other studies reported the mapping of this homoeologous gene on the short arms of chromosomes 2A and 2B (Simonetti et al. 1999; Dvorak et al. 2012; Faris et al. 2014a, b). It has been confirmed by different studies that wild emmer carries the primitive Tg^{2A} and Tg^{2B} alleles, whereas durum carries the domesticated tg^{2A} and tg^{2B} alleles (Simonetti et al. 1999; Dvorak et al. 2012; Faris et al. 2014a). However, the genotype of cultivated emmer for the *Tg* loci was not confirmed until Faris et al. (2014b) reported the mapping of Tg^{2A} and Tg^{2B} in a tetraploid RIL population known as BP025 developed from a cross between cultivated emmer and durum wheat. Cultivated emmer is known to be a rich source of genetic variation (Faris et al. 2014b). Therefore, by knowing the presence or absence of these free threshing governing genes in durum cultivars and germplasm, we can plan future breeding strategies for introgression of effective disease resistance genes from wild and cultivated emmer wheat accessions.

2.2. Stem rust life cycle

The stem rust causing fungus, *Puccinia graminis,* infects the stem, leaves and leaf sheaths (De wolf et al. 2011). Pathogen infestation prior to the heading stage can lead to major yield losses (De wolf et al. 2011). *Puccinia graminis* is biotrophic and heteroecious in nature, having two hosts (Singh et al. 2008). Bread wheat, durum wheat, barley and triticale act as primary hosts and *Berberis vulgaris* (common barberry), is considered as the most important alternative host (Singh et al. 2008).

The *P. graminis* life cycle consist of five stages; basidiospores (n), pycniospores (n), aeciospores (n+n), urediospores (n+n), and teliospores (2n) (Roelfs 1985; Leonard and Szabo 2005). These stages include both sexual (basidiospores, pycniospores, aeciospores, teliospores) and asexual phases (urediospores) (Roelfs 1985). Basidiospores produced by the germination of the teliospores are small, oval shaped and short lived in nature (Roelfs 1985). Basidiospores infect the leaves, stems and other parts of the alternative host *Berberis* (Roelfs 1985). Basidiospores lead to the formation of haploid, unicellular pycniospores (Roelfs 1985). Pycniospores act as sexual gametes and are involved in fertilization (Roelfs 1985). Fertilization forms the dikaryotic, cylindrical aeciospores. Aeciospores infect the gramineous host and produce the urediospores (Roelfs 1985). Urediospores are dikaryotic, oblong, and have the capability to travel long distances (Hirst and Hurst 1967; Luig 1977; Roelfs 1985). Large numbers of urediospores are produced by the asexual cycle (Roelfs 1985). The urediospores develop into blackish-brown, oblong, diploid, two-celled teliospores (Roelfs 1985). Teliospores have the capability to endure many environmental stresses (Roelfs 1985). They produce the basidiospores and repeat the cycle again (Roelfs 1985). The production of a large number of spores, the high rates of mutation and recombination, and the ability to travel long distances are

the major characteristics of the rust pathogens which enhance their capability to overcome barriers and lead to the evolution of new virulent races (Singh et al. 2008; Hulbert and Pumphrey 2014).

2.3. Stem rust virulence

Stem rust had been a significant disease in Africa, Australia, New Zealand, Europe, the Middle East, and Asia (excluding Central Asia) for thousands of years (Saari and Prescott 1985; Singh et al. 2008). Stem rust has been a threat to wheat production in the United States for hundreds of years (De wolf et al. 2011). Three epidemics were reported in the north central states in 1878, 1904 and 1916 (Roelfs 1978). The presence of the alternative host barberry in the north central states was the major cause of these epidemics until 1928 when barberry was removed from the region (Roelfs 1978). Thereafter, the pathogen overwintered in the southern states, which led to the survival of the rust pathogen (Roelfs 1978). A series of epidemics occurred in the states during the early 1930's and 1950's (Roelfs 1978; De wolf et al. 2011). A total of nine outbreaks of stem rust have been reported in the United States since 1904 (Paarlberg et al. 2014). An epidemic caused by race 56 in 1935 was the worst epidemic, and led to a greater than 50% loss in wheat production in North Dakota (56.5%) and Minnesota (51.6%) (Roelfs 1978).

Another epidemic occurred in the USA in 1953 and 1954 caused by race 15B (Roelfs 1978). Most of the varieties grown at that time were highly susceptible (Roelfs 1978). Because of this, the epidemic caused 35% and 80% yield loss to spring and durum wheat, respectively, in Minnesota and the Dakotas (Dubin and Brennan 2009; Paarlberg et al. 2014). This loss was estimated at nearly $3 billion (in 2009 prices) (Paarlberg et al. 2014; Milus et al. 2010). A more recent epidemic of stem rust in the 20th century occurred in the southeastern states in 1974, which caused 5-20% losses in yield (Roelfs 1978). Based on historical data, South Dakota, North

Dakota, Minnesota, Manitoba and Saskatchewan are under high risk for future stem rust outbreaks (Roelfs 1978).

Central India (1946-47), Mexico (1947-48) (Bajio region) and Chile (1951) wheat production has been devastated by stem rust, experiencing 20, 30 and 50% yield losses, respectively (reviewed in Dubin and Brennan 2009; Herrera-Foessel et al. 2014). In 1993 and 1994, a stem rust epidemic was reported in Ethiopia on the wheat variety "Enkoy" (Shank 1994; Singh et al. 2008). The Green Revolution resulted in combating stem rust with the slow rusting, adult plant resistance (APR) *Sr2* gene and a few other major genes transferred from hexaploid and tetraploid emmer wheat (McFadden 1930; Singh et al. 2008; Lopez-Vera et al. 2014). However, in 1998 the detection of *Pgt* race TTKSK, also known as Ug99, in Uganda alarmed the whole world due to its virulence against 80-90% of the worldwide wheat varieties and germplasm (Jin and Singh 2006; Fetch 2007; Singh et al. 2008; Lopez-Vera et al. 2014).

Ug99 virulence against the widely used and highly effective stem rust resistance gene *Sr31* poses a serious risk for wheat production in North Africa, the Middle East and southwest Asia due to favorable environmental conditions and cultivation of susceptible varieties (Singh et al. 2008; Periyannan et al. 2014a). In 2005, Ug99 race TTKSK was detected in Kenya and Ethiopia and in 2006, it was found in Sudan and Yemen (Wanyera et al. 2006; Singh et al. 2008; Sharma et al. 2013). In 2006 a new variant of Ug99, race TTKST, was found in Kenya, which carries virulence against the stem rust resistance genes *Sr24* and *Sr31* (Jin et al 2007; Singh et al. 2008). To date, thirteen variants of the Ug99 group, TTKSK, TTKSF, TTKST, TTTSK, TTKSP, PTKSK, PTKST, TTKSF+, TTKTT, TTKTK, TTHSK, PTKTK, and TTHST have been detected in 13 different African countries (Pretorius et al. 2010; Hale et al. 2013; Fetch et al. 2016; Table 2.1; http://rusttracker.cimmyt.org). These races belong to the Ug99 race group on the basis of a

common hypothetical ancestor, place of origin and simple sequence repeat analysis (SSR) (Park et al. 2011; Mukoyi et al. 2011; Pretorius et al. 2012; http://rusttracker.cimmyt.org). Among this Ug99 group, some races carry additional virulence against *Sr24*, *Sr36*, *SrTemp*, and *Sr9h* genes (http://rusttracker.cimmyt.org; Singh et al. 2015).

In addition to the Ug99 group, there are two additional stem rust races, TTTTF and TRTTF, that pose a threat to winter and durum wheat production, respectively (Jin 2005; Olivera et al. 2012). In 2003, stem rust race TTTTF was identified in Texas and Minnesota (Jin 2005). TTTTF differs from the Ug99 group on the basis of origin and virulence range. TTTTF is virulent on *Sr6, Sr10*, *Sr36* and *SrTmp*, which are important genes for winter wheat cultivars (Jin 2005). Stem rust race TRTTF, having virulence against *Sr9e* or *Sr13*, was detected in Ethiopia during 1988 and 1989 (Mengistu et al. 1991; Olivera et al. 2012). Along with that, TRTTF has virulence against the *Sr36, SrTmp* and $Sr1RS^{Amigo}$ (Olivera et al. 2012). Differences in the virulence range and morphology led to the differentiation of TRTTF from the Ug99 lineage. In durum wheat cultivars, stem rust resistance genes *Sr8b*, *Sr9e* and *Sr13* are frequently present. Out of these three only *Sr13* is effective against stem rust virulence in most of the wheat growing regions, with the exception of Ethiopia and India (Luig 1983; Bhavani et al. 2008; Qamar et al. 2009; Periyannan et al. 2014b). In 2013, another non-Ug99 lineage *Pgt* race, TKTTF, was detected in Ethiopia and Germany (Olivera et al. 2015, 2017) which led to 100% yield loss of the Ethiopian cultivar Digalu (Olivera et al. 2015; Turner et al. 2016). Together, Ug99 and non-Ug99 *Pgt* races carry a great potential threat to wheat production. In general, durum varieties and breeding lines from the United States, Canada, Egypt and the International Maize and Wheat Improvement Center (CIMMYT) have moderate to high levels of resistance to stem rust race TTKSK (Olivera et al. 2012; Pozniak et al. 2008; Singh et al. 2011). To prevent future yield

losses, there is need to introduce effective durable resistance genes in to current and future common bread and durum wheat cultivars.

2.4. High throughput genotyping

The ultimate goal of plant genetics is to improve the productivity and quality of cultivars (Moose and Mumm 2008). Molecular technologies decrease the time, labor, money and space needed to select new varieties with desirable traits. Single nucleotide polymorphisms (SNPs) are the most abundant class of markers, and they are codominant in nature (Rafalski 2002). SNPs have applicability in association studies, genotyping, diversity analysis and gene tagging (Rafalski 2002; Wang et al. 2014).With the innovation of next generation sequencing, SNP discovery has become more advanced (Berkman et al. 2012; Chia et al. 2012; Xu et al. 2012; Wang et al. 2014). Multiplexing thousands of SNPs on a single chip is the most advantageous feature. To date, SNP arrays have been developed for crops such as rice (44K), maize (50K) and wheat (9K, 90K, 660K, and 820K) (Wiedmann et al. 2008; Ganel et al. 2011; Zhao et al. 2011; Cook et al. 2012; Sim et al. 2012; Song et al. 2013; Wang et al. 2014, Jia and Zhao 2016; Winfield 2016). The development of an integrated genetic map consisting of 421,065 markers (Saintenac et al. 2013a) and the 90K iSelect genotypic assay (Wang et al. 2014) provide useful tools and information for the rapid mapping of genes. The development of a consensus map for stem rust resistance loci in wheat that includes stem rust resistance genes, QTLs, and molecular markers will play an important role in the identification of new *Sr* genes (Yu et al. 2014).

To speed the development of improved breeding lines there is need to use advanced molecular techniques (Varshney et al. 2014). Periyannan et al. (2013) cloned the *Sr33* gene introgressed from the diploid D-genome progenitor of hexaploid wheat *Ae. tauschii* (DD), which elicits a near immune response to Ug99 and its lineage. Saintenac et al. (2013b) cloned the stem

rust resistance gene *Sr35* derived from *T. monococcum* (A^mA^m); a close relative of the wheat A genome donor species *T. urartu* (AA). *Sr35* gives an intermediate level of resistance against Ug99 and other *Pgt* races (Saintenac et al. 2013b). Both genes have a coiled-coil-nucleotide binding–leucine-rich repeat (NB-LRR) structure (Saintenac et al. 2013b; Periyannan et al. 2013). Combining both genes with *Sr2* will give enhanced resistance against Ug99 and new *Pgt* races (Saintenac et al. 2013b; Periyannan et al. 2013). The use of cloned genes makes it easier to co-deploy multiple genes in a single breeding line by using marker-assisted selection (MAS). MAS plays an important role in the identification of desirable genotypes in a short time period with minimal cost (Ronald 2014). With the recent development of a fluorescence and gel-based SNP genotyping method known as semi-thermal asymmetric reverse PCR (STARP), it will be more convenient to screen a large number of plants in a short duration of time (Long et al. 2017). By developing STARP markers for existing and new *Sr* genes, it will become possible to expedite the co-deployment of multiple genes in a single breeding line using MAS.

Table 2.1. The different races under the Ug99 group, their year and place of detection

Race	Key virulence (+) or Avirulence (-)	Year of identification	Confirmed countries
TTKSK	*+Sr31*	1999	Uganda (1998/99) Kenya (2001), Ethiopia (2003), Sudan (2006), Yemen (2006), Iran (2007), Tanzania (2009), Eritrea (2012), Rwanda (2014), Egypt (2014)
TTKSF	*-Sr31*	2000	South Africa (2000), Zimbabwe (2009), Uganda (2012)
TTKST	*+Sr31, +Sr24*	2006	Kenya (2006), Tanzania (2009), Eritrea (2010), Uganda (2012), Rwanda (2014), Egypt (2014)
TTTSK	*+Sr31, +Sr*36	2007	Kenya (2007), Tanzania (2009), Ethiopia (2010), Uganda (2012), Rwanda (2014)
TTKSP	*-Sr31, +Sr24*	2007	South Africa (2007)
PTKSK	*+Sr31, -Sr21*	2007	Uganda [(1998/9)?], Ethiopia (2007), Kenya (2009), Yemen (2009)
PTKST	*+Sr31, +Sr24, -Sr21*	2008	Ethiopia (2007), Kenya (2008), South Africa (2009), Eritrea (2010), Mozambique (2010), Zimbabwe (2010)
TTKSF+	*-Sr31, +Sr9h*	2012	South Africa (2010), Zimbabwe (2010)
TTKTT	*+Sr31, +Sr24, +SrTmp*	2015	Kenya (2014)
TTKTK	*+Sr31, +SrTmp*	2015	Kenya (2014), Egypt (2014), Eritrea (2014), Uganda (2014), Rwanda (2014)
TTHSK	*+Sr31, -Sr30*	2015	South Africa (2007)
PTKTK	*+Sr31, -Sr21, +SrTmp*	2015	Kenya (2014)
TTHST	*+Sr31, -Sr30, +Sr24*	2015	Kenya (2013)

Source-http://rusttracker.cimmyt.org

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3. GENETIC ANALYSIS OF THRESHABILITY AND OTHER SPIKE TRAITS IN THE EVOLUTION OF CULTIVATED EMMER TO FULLY DOMESTICATED DURUM WHEAT

3.1. Introduction

During the Agricultural Revolution, numerous plants and animals were domesticated at a rapid pace alongside the rise of human civilizations (see Faris 2014 for review). Emmer wheat was one of the Neolithic founder crops, and its descendants now provide about 20% of the total food calories consumed by humans. Among today's domesticated wheat species, hexaploid bread wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD) and tetraploid durum wheat [*T. turgidium* ssp. *durum* (Desf.) Husnot, $2n = 4x = 28$, AABB] account for 95% and 5% of the worldwide wheat production, respectively (Peng et al. 2011). Durum wheat, which is mainly used for making pasta and other semolina-based products, is grown on about 17 million hectares worldwide (Hu et al. 2015), with Europe, the upper Great Plains of the U.S., and the Mediterranean regions as the major producers (http://www.ndwheat.com/uploads/resources/546/world-web-charts.pdf).

Modern durum and bread wheat varieties differ from their wild ancestors due to the acquisition of traits contributing to the domestication syndrome including the loss of a natural seed dispersal mechanism (shattering), reduction of seed dormancy, an increase in the size and number of seeds, and naked (free-threshing) grains (Simons et al. 2006; Peng et al. 2011; Faris et al. 2014a). This evolutionary trail consisted of multiple steps that provide insights into breeding and selection strategies adapted by the ancient farmers, which ultimately shaped modern agriculture (Doebley et al. 2006). A deeper understanding of these steps may allow researchers to

further improve modern cultivars through the identification and reintroduction of rare but beneficial alleles from wild wheat relatives.

Wild emmer [*T. turgidium* ssp. *dicoccoides* (Körn.) Thell (2*n* = 4*x* = 28, AABB)] is an allotetraploid that originated from an amphiploidization event that occurred around 0.5 MYA ago (Huang et al. 2002; Chalupska et al. 2008) between two diploid species, *T. urartu* Tumanian ex Gandylian (2*n* = 2*x* = 14, AA) (Dvorak et al. 1993) and a close relative of *Aegilops speltoides* ssp. *lingustica* Tausch (2*n* = 2*x* = 14, SS) (see Faris 2014 for review). Wild emmer wheat has a brittle rachis and hulled grains; traits which allow it to thrive as a wild species, but are not conducive to cultivation. The evolution of cultivated emmer [*T. turgidium* ssp. *dicoccum* (Schrank) Schübl, 2*n* = 4*x* = 28, AABB] from wild emmer was led by the loss of the brittle rachis trait (and other modifications), which prevented spikelet shattering making it easy for farmers to harvest the grains upon maturity without the spikelets dropping to the ground prematurely and becoming lost. In tetraploid wheat, loci governing the brittle rachis (*Br*) have been reported to reside on chromosomes 2A, 3A, and 3B (Peng et al. 2003; Peleg et al. 2011; Li and Gill 2006; Nalam et al. 2006; Thanh et al. 2013). Recent cloning of the *Br* genes on chromosomes 3A and 3B indicates that recessive mutations were required in both genes to confer a nonshattering spike (Avni et al. 2017). However, the grains of cultivated emmer were not free-threshing making post-harvest processing yet a laborious task (see Faris 2014 for review; Tzarfati et al. 2013).

Modern durum wheat differs from cultivated emmer by having free-threshing grains (also known as naked, or non-hulled) (see Faris 2014 for review, Faris et al. 2014a, c). Durum wheat first appeared in the archeological record about 7,000 years ago (Hillman 1978). Genetically, the evolution of the free-threshing trait in durum was due to mutations in three major genes,

including *q* on chromosome arm 5AL and homoeologous tenacious glume (*Tg*) genes on chromosome arms 2AS and 2BS (Simonetti et al. 1999; Dvorak et al. 2012; Faris 2014; Faris et al. 2014a, c). Therefore, mutations in the *q* and *Tg* loci must have occurred prior to this time period.

The *q* gene is a member of the *APTETALA2* (*AP2*) family of transcription factors, and it has effects on multiple domestication and agronomic traits including threshability, rachis fragility, plant height, spike length, glume morphology, and heading time (Unrau et al. 1950; Sears 1956; Muramatsu 1963, 1979, 1985, 1986; Kato et al. 1999, 2003; Faris and Gill 2002; Faris et al. 2003; Simons et al. 2006; Zhang et al. 2011; Faris 2014). It was recently demonstrated that a single point mutation in the microRNA172 binding site of the *q* gene exon 9 results in reduced microRNA172 binding, which leads to higher transcription levels (the *Q* allele), which results in a more compact spike with free-threshing seed (Debernardi et al. 2017; Greenwood et al. 2017). Therefore, the mutation that resulted in the *Q* allele, which is present in virtually all modern common and durum wheat varieties (Muramatsu 1985; Faris et al. 2014b), could be considered a gain-of-function mutation that had a substantial impact on the rise of modern agriculture.

The first *Tg* locus was identified and described in the D-genome progenitor [*Ae. tauschii* Coss. ($2n = 2x = 14$, DD genome)] of hexaploid wheat (Kerber and Dyck 1969) on chromosome arm 2DS (Kerber and Rowland 1974), and it was reported to be epistatic to *Q* because synthetic hexaploids derived from durum (*QQ* genotype) × *Ae. tauschii* (*TgTg* genotype) were found to be nonfree-threshing. *Tg* loci were not discovered on the other homoeologous group 2 chromosomes until Simonetti et al. (1999) reported a QTL associated with threshability on 2BS in a wild emmer × durum wheat population. Since then, the presence of a *Tg* locus on 2BS in

wild emmer was confirmed (Faris et al. 2014a), and evidence of the presence of a *Tg* locus on 2AS in the hexaploid relative *T. aestivum* ssp. *spelta* (2*n* = 6*x* = 42, AABBDD genomes) was provided by Dvorak et al. (2012). However, it was not until the study conducted by Faris et al. (2014c), who employed a durum × cultivated emmer population involving the durum variety Ben and the cultivated emmer accession PI 41025 (referred to as the BP025 population), that is was shown that cultivated emmer harbored primitive *Tg* alleles on both 2AS and 2BS as well as the primitive *q* allele. Faris et al. (2014c) therefore proposed that mutations in the genes governing the free-threshing character, i.e. Tg^{2A}, Tg^{2B}, and *q*, occurred during the evolution of cultivated emmer to modern durum wheat.

The first objective of the current work was to evaluate the effects of the major tetraploid domestication loci Tg^{2A}, Tg^{2B}, and *q* in a different durum × cultivated emmer biparental population to validate the findings reported by Faris et al. (2014c), and to gain further understanding of their effects on threshability and other domestication traits. Our second objective was to identify QTL associated with other domestication and agronomic traits such as rachis fragility, spike length, spikelets per spike, spike compactness, days to heading, and plant height.

3.2. Material and methods

3.2.1. Plant material

A tetraploid wheat population consisting of 256 recombinant inbred lines (RILs) (F_7 generation) was developed from a cross between the durum wheat line Rusty (Klindworth et al. 2006) and the cultivated emmer accession PI 193883 by using the single-seed descent method. This population is hereafter referred to as the RP883 population. Of the 256 total RILs, 190 were randomly selected for phenotyping and genotyping.

3.2.2. Phenotyping

The RP883 population and parents were phenotyped for a total of seven traits including days to heading (DTH), plant height (HT), spike length (SL), number of spikelets per spike (SPS), spike compactness (SC), threshability (TH), and rachis fragility (RF). The study was conducted using completely randomized block designs for two greenhouse seasons (Fall 2015 and Fall 2016). Greenhouse growth conditions consisted of a temperature of 21°C and a 16-hour photoperiod. In both seasons, one seed per RIL was planted in a six-inch clay pot containing soil supplemented with fertilizer to create a single experimental unit. A total of three replications were included in the Fall 2015 experiment and two replications in the Fall 2016 experiment.

To measure DTH, days were counted between seed sowing and emergence of the first head. HT was measured in centimeters after the plants were fully matured. The other traits including SL, SPS, SC, TH, and RF were measured after harvesting by calculating the mean of four main spikes for each experimental unit. SL was measured in centimeters and for SPS all the spikelets (including sterile ones) were counted. SC was calculated by dividing the SL by SPS for each spike. TH and RF were scored using the 1-4 scales described in Faris et al. (2014a, c), where; 1 = free-threshing, tough rachis and 4 = non-free-threshing, fragile rachis.

3.2.3. Statistical analysis

Phenotypic data of both seasons for all traits was statistically analyzed by using PROC GLM in the SAS program version 9.3 (SAS institute 2011). First, the phenotypic data was analyzed for normal distribution. The data was further analyzed for homogeneity of variances within and between the two seasons by using Bartlett's test (Snedecor and Cochran 1989) for the normally distributed data and Levene's test (Levene 1960) for the data that deviated from normal distribution. Each trait mean for both seasons was used to calculate the trait value range and

standard deviation. To identify significant differences between the means of the RILs for each trait, Fisher's least significant difference (LSD) was calculated at $\alpha = 0.05$. Correlation analysis was conducted between the traits by using the mean of RILs and significant *p* value was assigned for each correlation. The mean of the RILs were used to develop a histogram representing the frequency distribution of RILs for each trait.

3.2.4. Genotyping and linkage map generation

DNA of the 190 RILs was extracted using the ammonium acetate method (Pallota et al. 2003). The DNA was dissolved in 100 µl of water and quantified using a Nanodrop spectrophotometer ND-1000 (Thermo Scientific, Wilmington, DE). RILs were genotyped using the 90K iSelect array (Illumina, San Diego, CA) on an Illumina iScan instrument (Illumina, San Diego, CA). Samples were clustered into separate groups on the basis of hybridization intensities. These clusters were analyzed by using the GenomeStudio (Illumina, San Diego, CA) polyploidy clustering module based on "ordering points to identify the clustering structure (OPTICS)" clustering algorithm (Ankerst et al. 1999). Single nucleotide polymorphism (SNP) loci were assigned to chromosome locations based on the consensus map developed by Wang et al. (2014).

Chromosome-specific microsatellite (simple sequence repeat; SSR) markers were also used to genotype the RP883 population and were selected from a microsatellite 'core' set (Sorrells et al. 2011). The SSR primer sets were selected from WMC (Wheat Microsatellite Consortium) (Somers et al. 2004), GWM (Gatesleben Wheat Microsatellite) (Röder et al. 1998a, b), CFA (Clermont Ferrrand A genome) (Sourdille et al. 2003) BARC (Beltsville Agriculture Research Center) (Song et al. 2005), and HBG (Torada et al. 2006) libraries. The *Q* gene functional marker *Xfcp650* (Simons et al. 2006) was placed on the map as well. For SSRs,

polymerase chain reaction (PCR) was applied according to Röder et al (1998b), and PCR products were electrophoresed on 6% nondenaturing or 8% denaturing polyacrylamide gels, stained with Gelred™ nucleic acid stain (Biotium Corporate, Hayward, CA), and scanned with a Typhoon 9410 variable mode imager (GE healthcare Biosciences, Waukesha, WI).

Linkage analysis was performed using the program MapDisto 1.8.2.1 (Lorieux 2012). Linkage groups were initially identified using the command "find groups" with a logarithm of odds (LOD) = 3.0 and an Rmax value = 3.0 for each chromosome. This command divided the initial sequences into different groups with regard to their two-point recombination fractions, and LOD scores for the linkage. To determine the best order of markers, the 'order sequence,' 'check inversions,' 'ripple order,' and 'drop locus' commands were used. Map distances, measured in centimorgans (cM), were calculated using the Kosambi mapping function (Kosambi 1943). For each locus, segregation distortion was calculated for the deviation from 1:1 ratio by using the segregation χ^2 test at $P = 0.05$. Marker loci with segregation ratios that deviated significantly from a 1:1 ratio were given the symbol from "*" to "*****", where "*****" were assigned to the most deviated loci with $P <$ 1e-05 and "*" were the least deviated loci with $P < 0.05$ (Lorieux 2012).

3.2.5. QTL analysis

The linkage maps and phenotypic means for each trait were evaluated for significant marker-trait associations using the quantitative trait loci (QTL) analysis software program QGENE version 4.3.10 (Joehanes and Nelson 2008). QTL associated with agronomic and domestication traits were identified by regressing the phenotypic data on genotypic data through single-trait multiple interval mapping (MIM). For each trait permutation test, 1000 iterations were carried out to determine the significant LOD threshold. A critical LOD value cut off of 4.2

at $P \geq 0.05$ was used for the whole-genome QTL analysis. The coefficient of determination (R^2) was used to determine the percent of phenotypic variation explained by each QTL.

3.2.6. Genotypic classes associated with threshability

Threshability means for all possible genotypic classes based on allelic states at the *Tg*^{2A}, *Tg*^{2B}, and *Q* loci were calculated to further evaluate the effects of the three genes. For the *Tg*^{2A} and *Tg*^{2B} loci, flanking markers were used to select the RILs carrying the *Tg* and *tg* alleles from PI 193883 and Rusty, respectively. RILs carrying *Q* from Rusty or *q* from PI 193883 on chromosome 5A were identified using the *Xfcp650* marker. The mean threshability scores of the selected RILs were used to calculate the LSD for the genotypic classes.

3.3. Results

3.3.1. Linkage maps

The whole-genome linkage maps of the RP883 population were assembled into 14 linkage groups corresponding to the 14 durum chromosomes and were comprised of 65 SSR and 9281 SNP markers (Table 3.1, Supplementary file 1). Chromosome 4B was the shortest linkage group at 130.5 cM in length, and the largest linkage group was 7A at 229.7 cM. Density ranged from 0.9 to 1.9 cM/locus among linkage groups, with a genome-wide density of 1.4 cM/marker locus. The B genome linkage groups were more dense compared to the A genome. The percentage of markers with distorted segregation ratios varied among chromosomes and ranged from 0% on chromosomes 4B and 5B to 52.8% on chromosome 3B.

Table 3.1 Summary of the linkage groups and genome mapping parameters for the RP883 population

Chromosome	SSR	SNP	Total markers	Loci	Length	cM/loci	% distorted markers
1A	5	737	742	133	151.3	1.1	0.1
1B	4	816	820	157	160.7	1.0	11.0
2A	5	487	492	90	174.9	1.9	8.9
2B	6	1000	1006	186	165.1	0.9	44.1
3A	4	485	489	119	190.7	1.6	15.3
3B	4	712	716	171	196.9	1.2	52.8
4A	3	536	539	115	200.6	1.7	31.4
4B	3	378	381	78	130.5	1.7	0.0
5A	6	501	507	128	210.5	1.6	3.6
5B	7	963	970	164	194.2	1.2	0.0
6A	6	656	662	103	139.3	1.4	11.3
6B	4	666	670	128	133.7	1.0	34.9
7A	4	603	607	145	229.7	1.6	9.6
7B	4	741	745	117	162.1	1.4	0.5
A genome	33	4005	4038	833	1297.0	1.6	10.8
B genome	32	5276	5308	1001	1143.2	1.2	21.7
Total	65	9281	9346	1834	2440.2	1.4	17.0

3.3.2. Trait analysis

The homogeneity of variance analyses indicated that variances between the Fall 2015 and Fall 2016 experiments were not significantly ($P < 0.05$) different for any of the traits with the exception of DTH and RF. Therefore, the data from the two seasons for DTH and RF were evaluated separately for statistical and QTL analysis for these two traits. For DTH, Fall 2015 and Fall 2016 data were designated as DTHa and DTHb, respectively. During both seasons, Rusty took 29 and 24 days longer to head, respectively, compared to PI 193883 (Fig. 3.1, Table 3.2). The population mean (54.9 days) and frequency distribution (40-60 days) suggested that increased DTH in the RILs was contributed by Rusty.

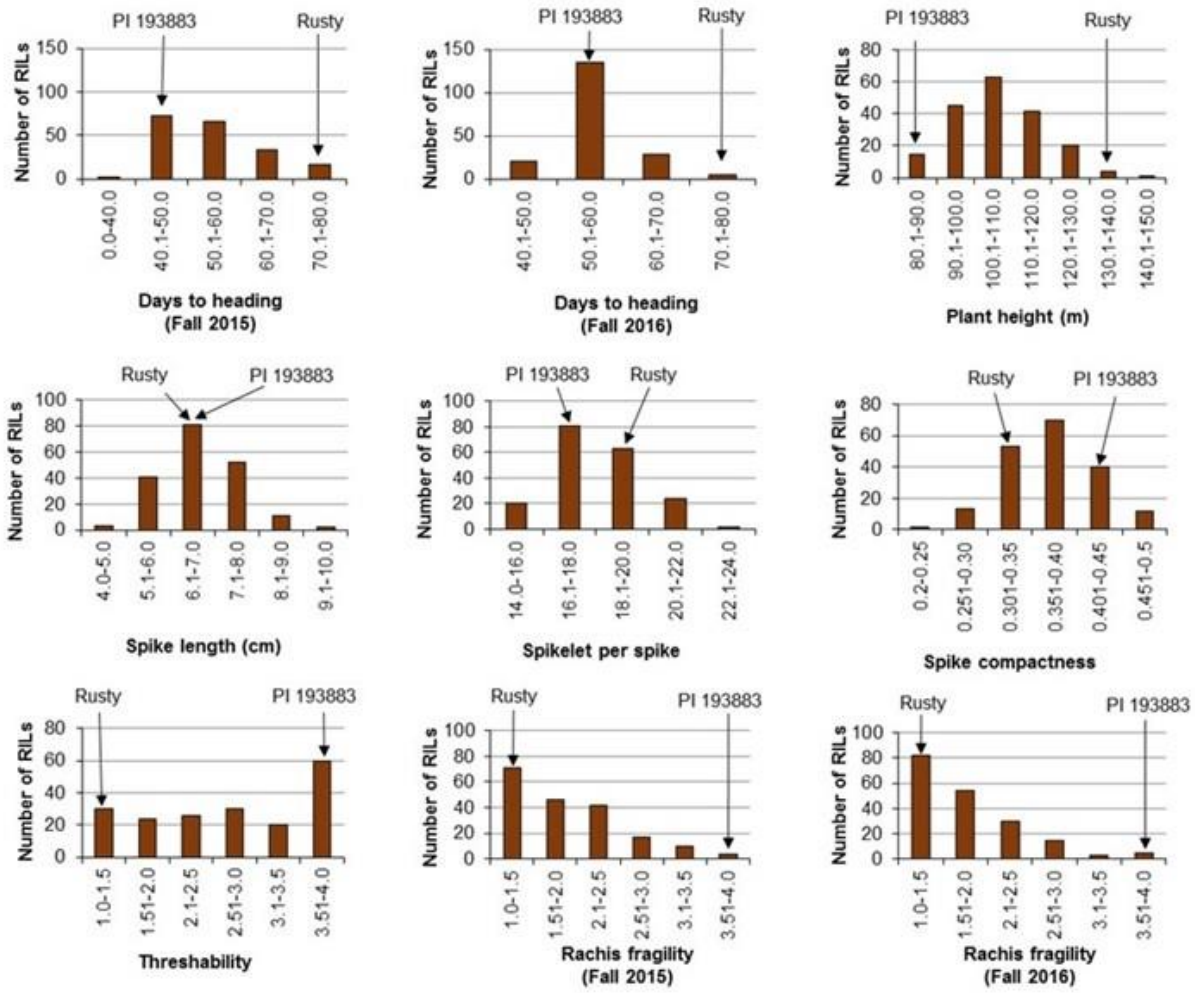

Fig. 3.1. Histograms for a total of seven phenotypic traits: days to heading Fall 2015, days to heading Fall 2016, plant height, spike length, spikelets per spike, spike compactness, threshability, rachis fragility Fall 2015, and rachis fragility Fall 2016 evaluated in the RP883 population for two greenhouse seasons. Mean trait values of both seasons were used for all traits except for days to heading and rachis fragility.

On average, Rusty was 52 cm taller than PI 193883 (Fig. 3.1, Table 3.2). HT for the RP883 population ranged from 80.7-146.3 cm with a mean of 106.4 cm, which was the average HT of both parents indicating that they harbor multiple genes governing HT.

Rusty and PI 193883 were not significantly different for SL (Table 3.2). However, the population mean ranged from 4.3 to 9.3 cm with Rusty (6.2 cm) and PI 193883 (6.7 cm) mean values being similar to the population mean (6.7 cm). Therefore, Rusty and PI 193883 carry

different genes that influence SL (Table 3.2). SPS mean values for Rusty, PI 193883, and the RP883 population were 18.9, 16.7 and 18.1, respectively (Fig. 3.1, Table 3.2). PI 193883 had a less compact spike compared to Rusty (Fig. 3.1, Table 3.2).

Table 3.2. Simple Stat and LSD (α = 0.05) analysis for Rusty, PI 193883, and the RP883 population for days to heading Fall 2015 (DTHa), days to heading Fall 2016 (DTHb), plant height (HT), spike length (SL), spikelet per spike (SPS), spike compactness (SC), threshability (TH), rachis fragility Fall 2015 (RFa), and rachis fragility Fall 2016 (RFb)

Trait	Parents[a]		Population				
	Rusty	PI 193883	Minimum	Maximum	Mean	Standard deviation	LSD (α = 0.05)
DTHa	71.0*	41.7*	38.7	79.3	54.9	9.1	8.53
DTHb	74.5*	51.0*	47.0	74.0	56.0	5.3	7.37
HT	132.8*	81.1*	80.7	146.3	106.4	12.1	12.10
SL	6.2	6.7	4.3	9.3	6.7	0.9	0.68
SPS	18.9*	16.7*	14.2	22.2	18.1	1.6	1.75
SC	0.3*	0.4*	0.2	0.5	0.4	0.1	0.04
TH	1.1*	4.0*	1.0	4.0	2.7	1.0	1.01
RFa	1.2*	3.8*	1.0	4.0	1.9	0.7	1.24
RFb	1.3*	4.0*	1.0	4.0	1.8	0.7	1.18

[a]The significant trait values for parents Rusty and PI 193883 based on the LSD (α = 0.05) are indicated by an *asterisk* (*).

Upon mechanical threshing, the rachises of Rusty spikes generally remained intact and the glumes, lemma and palea tended to break at the base of the florets, which allowed easy liberation of the kernels (Fig. 3.2). On the contrary, the rachises of PI 193883 spikes disarticulated and the spikelets remained intact prohibiting liberation of the kernels. Therefore, Rusty and PI 193883 were significantly different from each other for TH with mean values of 1.1 and 4.0, respectively (Table 3.2, Fig. 3.1, 3.2). TH values for the RP883 population ranged from 1.0 to 4.0, and had an overall average of 2.7 (Table 3.2, Fig. 3.1) suggesting that multiple genes derived from PI 193883 were responsible for conferring the non-free-threshing character.

RFa and RFb designations were used for the Fall 2015 and Fall 2016 RF data, respectively. Average RF scores for Rusty and PI 193883 were 1.2 and 3.8 for RFa, and 1.3 and 4.0 for RFb, respectively, indicating significant differences in the RF trait between the parents

(Table 3.2, Fig. 3.1). The RP883 population had average RF scores of 1.9 and 1.8 for the two seasons, which were not significantly different from the average RF scores of Rusty.

Fig. 3.2. Spikes of the durum wheat line Rusty and cultivated emmer wheat accession PI 193883 after threshing.

The results of the correlation analysis between the seven traits are given in Table 3.3. DTHa had a positive correlation with HT, SPS, and RFb, and a strong negative correlation with SC. DTHb was also positively correlated with SPS and RFb; however, it was negatively correlated with SC, TH, and SL. Increase in HT was directly influenced by an increase in the phenotypic values of DTHa, SL, SPS, SC, and TH. Similarly, SL was strongly correlated with HT, SPS, SC, and TH, and weakly correlated with DTHb.

Table 3.3 Pearson's correlation values and their statistical significance between the mean values of phenotypic traits: days to heading Fall 2015 (DTHa), days to heading Fall 2016 (DTHb), plant height (HT), spike length (SL), spikelet per spike (SPS), spike compactness (SC), threshability (TH), rachis fragility Fall 2015 (RFa), and rachis fragility Fall 2016 (RFb)

	DTHa	DTHb	HT	SL	SPS	SC	TH	RFa
DTHb	0.66***							
HT	0.35***	0.05						
SL	0.02	-0.16*	0.34***					
SPS	0.48***	0.26***	0.22**	0.35***				
SC	-0.30***	-0.31***	0.18*	0.76***	-0.33***			
TH	-0.07	-0.23**	0.18*	0.47***	-0.05	0.50***		
RFa	0.03	0.06	-0.02	-0.10	-0.02	-0.09	-0.24***	
RFb	0.15*	0.21**	-0.13	-0.08	0.06	-0.11	-0.35***	0.57***

$*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$

SPS values were positively correlated with DTH, HT, and SL and inversely with SC. This indicates that, with an increase in SPS, the spike became more compact and shorter in

length. SC was positively correlated with SL, TH, and HT, and negatively correlated with DTHa and DTHb. The high positive correlation between TH, SL, and SC showed that as the spike became longer and less compact, it became more difficult to thresh. In both seasons, RF was negatively correlated with TH, which indicates that as the spike became more non-free-threshing, the rachis became more fragile.

3.3.3. QTL analysis

Eleven QTL on eight chromosomes (1A, 1B, 2A, 2B, 4A, 4B, 5A, and 7B) associated with agronomic and domestication traits were identified in the RP883 population (Fig. 3.3, Table 3.4). For DTHa and DTHb, two common QTL were identified on chromosome arms 1AL and 5AL, and designated as *QEet.fcu-1A* and *QEet.fcu-5A*, respectively (Fig. 3.3, Table 3.4). For DTHa, *QEet.fcu-1A* had a LOD value of 14.4 and explained 13.3% of the variation. The *QEet.fcu-5A* QTL located in the *Vrn-A1* region vicinity had a LOD value of 23.0 and explained 33.6% of the variation. In addition, a QTL designated *QEet.fcu-7B* on chromosome arm 7BS had a LOD value of 4.4 and explained 4.2% of the variation (Fig. 3.3, Table 3.4). For DTHb, *QEet.fcu-1A* and *QEet.fcu-5A* had LOD values of 7.3 and 5.9, and explained 11.9 and 9.2% of the variation, respectively (Fig. 3.3, Table 3.4).

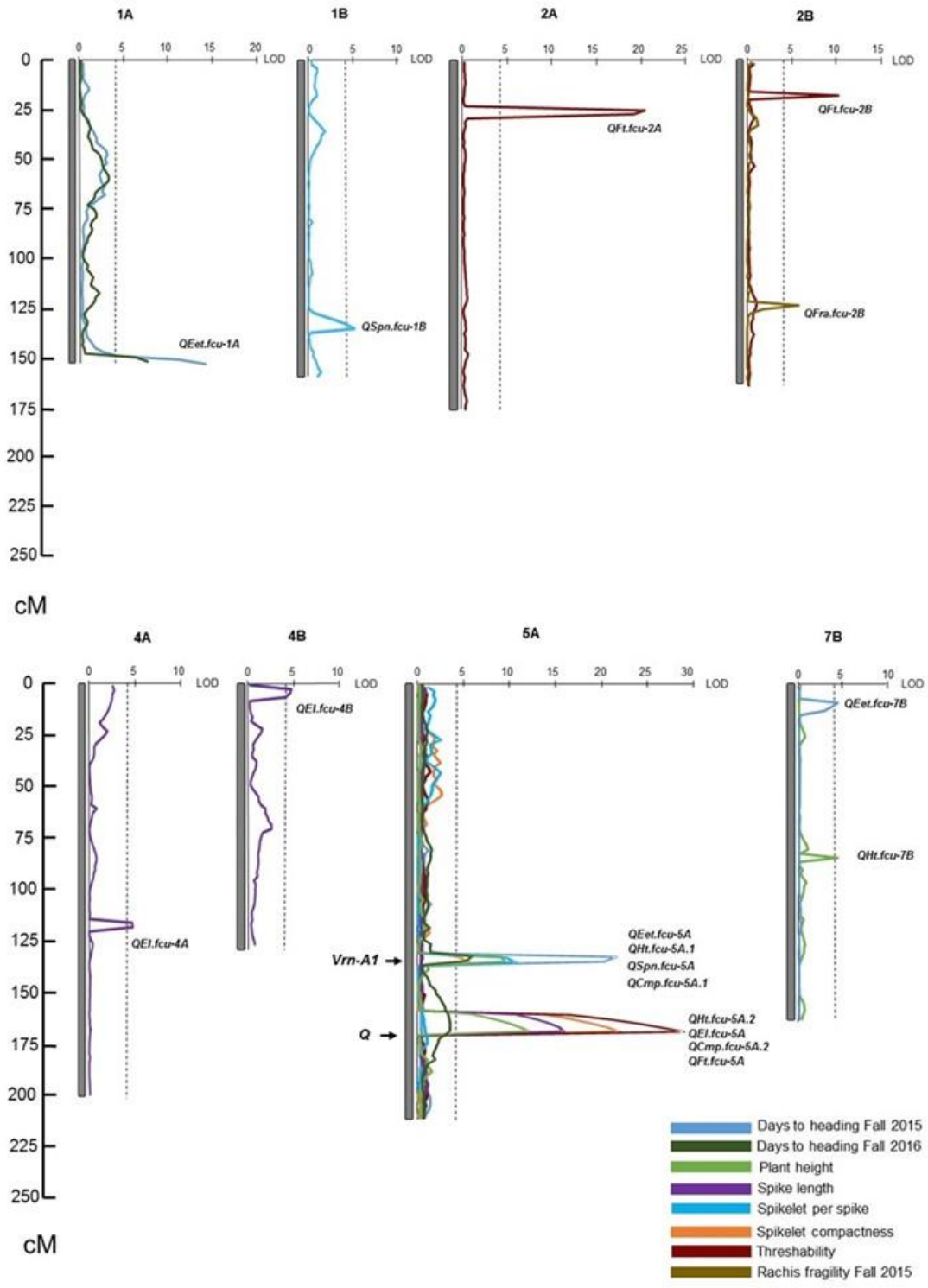

Fig. 3.3. Graphical representation of 11 QTL located on eight different chromosomes of the tetraploid wheat genome associated with a total of seven agronomic and domestication traits evaluated in the RP883 population. The known *Vrn-A1* and *Q* regions are indicated in *bold.*

Three QTL associated with HT were identified (Fig. 3.3, Table 3.4). *QHt.fcu-5A.1* (LOD = 9.9) and *QHt.fcu-7B* (LOD = 4.8) had effects for increased HT from Rusty and explained 9.8 and 7.5% of the phenotypic variation, respectively. The third QTL, *QHt.fcu-5A.2*, derived effects for increased HT from PI 193883. This QTL had a LOD value of 12.7 and explained 14.4 % of the variation. *QHt.fcu-5A.2* peaked at the functional *Q* locus marker *Xfcp650*, which indicates that increase in HT was contributed by the *q* allele derived from PI 193883. Additive values of the QTL showed that both parents carry genes for HT, which supports the results concluded from the trait analysis.

Three QTL associated with SL were identified on the chromosome arms 4AL, 4BS, and 5AL, and these QTL were designated as *QEl.fcu-4A*, *QEl.fcu-4B*, and *QEl.fcu-5A*, respectively (Fig. 3.3, Table 3.4). LOD values for these QTL ranged from 4.9-16.1 and they explained from 6.5 to 24.5% of the variation. For QTL *QEl.fcu-4A* and *QEl.fcu-5A*, an increase in SL was conferred by PI 193883 alleles, whereas for *QEl.fcu-4B* it was derived from Rusty. The effects of *QEl.fcu-5A* on SL were due to the *Q* locus.

For SPS, two QTL derived from Rusty were identified on chromosome arms 1BL and 5AL at positions 136 and 134 cM, and designated as *QSpn.fcu-1B* and *QSpn.fcu-5A*, respectively (Fig. 3, Table 4). These QTL had LOD values of 5.1 and 11.4, and explained 6.0 and 18.0% of the variation, respectively.

Table 3.4. Summary of the QTL detected in the RP883 population during two greenhouse seasons for seven agronomic and domestication traits

Trait[a]	QTL	Chromosome	Putative gene[b]	Position (cM)	LOD	$R^2 \times 100$	Marker interval	Additive effects[c]
DTHa	*QEet.fcu-1A*	1A	*ELF3*	150	14.4	13.3	*IWB36490-IWB45352*	3.93
	QEet.fcu-5A	5A	*Vrn-A1*	134	23.0	33.6	*IWB56489-IWA2350*	5.82
	QEet.fcu-7B	7B	*Ft-B1*	8	4.4	4.2	*IWB3164-IWA1089*	2.14
DTHb	*QEet.fcu-1A*	1A	*ELF3*	150	7.3	11.9	*IWB36490-IWB45352*	1.96
	QEet.fcu-5A	5A	*Vrn-A1*	134	5.9	9.2	*IWB56489-IWA2350*	1.83
HT	*QHt.fcu-5A.1*	5A	*Vrn-A1*	134	9.9	9.8	*IWB56489-IWA2350*	5.16
	QHt.fcu-5A.2	5A	*Q*	168	12.7	14.4	*IWB73761-Xfcp650*	-5.92
	QHt.fcu-7B	7B	-	82	4.8	7.5	*IWB73443-IWB23834*	3.38
SL	*QEl.fcu-4A*	4A	-	118	4.9	9.0	*IWB9196-IWB47072*	-0.26
	QEl.fcu-4B	4B	-	2	4.9	6.5	*IWB34708-IWA8109*	0.25
	QEl.fcu-5A	5A	*Q*	168	16.1	24.5	*IWB73761-Xfcp650*	-0.49
SPS	*QSpn.fcu-1B*	1B	-	136	5.1	6.0	*IWB8840-IWB64651*	0.49
	QSpn.fcu-5A	5A	*Vrn-A1*	134	11.4	18.0	*IWB56489-IWA2350*	0.77
SC	*QCmp.fcu-5A.1*	5A	*Vrn-A1*	134	5.7	15.9	*IWB56489-IWA2350*	-0.01
	QCmp.fcu-5A.2	5A	*Q*	168	22.9	41.5	*IWB73761-Xfcp650*	-0.03
TH	*QFt.fcu-2A*	2A	Tg^{2A}	24	21.1	22.8	*IWB60348-IWB1365*	-0.48
	QFt.fcu-2B	2B	Tg^{2B}	16	10.2	7.0	*IWB4953-IWB54956*	-0.37
	QFt.fcu-5A	5A	*Q*	168	29.0	32.5	*IWB73761-Xfcp650*	-0.59
RFa	*QFra.fcu-2B*	2B	-	124	5.8	13.1	*IWB22526-Xwmc332*	-0.23

[a]Days to heading Fall 2015 (DTHa), days to heading Fall 2016 (DTHb), plant height (HT), spike length (SL), spikelet per spike (SPL), spike compactness (SC), threshability (TH), and rachis fragility Fall 2015 (RFa).
[b]A *dash* (-) represents that gene associated with QTL is not known.
[c]A positive additive value indicates that higher scores for the trait were derived from Rusty, and a negative value indicates they were derived from PI 193883.

Because SC was calculated based on SL and SPS, it was expected that QTL associated with SL and SPS would also be associated with SC. Indeed, two QTL on chromosome arm 5AL, designated as *QCmp.fcu-5A.1* (134 cM) and *QCmp.fcu-5A.2* (168 cM), which overlapped with *QSpn.fcu-5A* and *QEl.fcu-5A*, respectively, were associated with SC (Fig. 3.3, Table 3.4). *QCmp.fcu-5A.1* had a LOD value of 5.7 and explained 15.9% of the variation, and *QCmp.fcu-5A.2*, which peaked at *Xfcp650(Q),* had a LOD value of 22.9 and explained 41.5% of the variation.

Three QTL associated with TH were identified on the chromosome arms 2AS, 2BS, and 5AL, and designated as *QFt.fcu-2A* (24 cM), *QFt.fcu-2B* (16 cM), and *QFt.fcu-5A* (168 cM), respectively (Fig. 3.3, Table 3.4). These QTL had LOD values of 21.1, 10.2, and 29.0, and explained 22.8, 7.0, and 32.5% of the variation, respectively. At each of the three loci, the non-free-threshing alleles were contributed by PI 193883. *QFt.fcu-5A* coincided with the *Q* locus at marker *Xfcp650(Q)*.

For RF, only the data from Fall 2015 revealed a significant QTL, which was located on chromosome arm 2BL and explained 13.1% of the variation (Fig. 3.3, Table 3.4). This QTL, designated *QFra.fcu-2B,* had a LOD value of 5.8, and its effects on increasing fragility of the rachis were derived from PI 193883 (Table 3.4).

3.3.4. Coincident QTL

The two QTL regions on chromosome arm 5AL at positions 134 and 168 cM were associated with multiple traits (Fig. 3.2, Table 3.4). The first QTL region, which was positioned at the marker interval *IWB56489-IWA2350* at 134 cM, was associated with traits DTH, HT, SPS, and SC. Comparisons with maps published by Zou et al. (2017) suggest that this region most likely corresponds to the vernalization locus *Vrn-A1,* which is responsible for spring vs. winter

growth habitat determination and is also known to effect HT (Kato et al. 1999; Yan et al. 2003; Kamran et al. 2014). The second 5A QTL, which was positioned at 168 cM, was significantly associated with HT, SL, SC, and TH. The *Q* gene-specific marker *Xfcp650(Q)* (Simons et al. 2006) defined the peak of this region thus validating the pleiotropic effects of *Q* (Faris et al. 2014c; Simons et al. 2006).

3.3.5. Analysis of genotypic classes for threshability

The RP883 population genomic data were grouped into eight genotypic classes for the three TH QTL on 2AS, 2BS, and 5AL based on whether or not they carried domestication alleles from Rusty or primitive alleles from PI 193883. The effects of the 2AS and 2BS QTLs were presumed to be due to homoeologous *Tg* loci, where the domestication alleles are *tg* and the primitive alleles are *Tg* (Faris et al. 2014c). The genotypes of these loci were inferred using flanking markers including *IWB60348* and *IWB1365* for *QFt.fcu-2A* (Tg^{2A}) and *IWB4953* and *IWB54956* for *QFt.fcu-2B* (Tg^{2B}). The genotypes at the 5A QTL, which coincided with the *Q* locus, were determined based on the marker genotypes at *Xfcp650(Q)*, which detects an SSR within the *Q* gene (Simons et al. 2006).

The TH mean of the eight classes ranged from 3.81 for the non-free-threshing primitive genotype ($Tg^{2A}Tg^{2B}q$) to 1.31 for the free-threshing domesticated genotype ($tg^{2A}tg^{2B}Q$) (Table 3.5). Compared to the PI 193883 parental genotype ($Tg^{2A}Tg^{2B}q$), lines with Rusty alleles for either tg^{2A} or tg^{2B} were not easier to thresh. However, lines with the *Q* allele from Rusty but PI 193883 alleles at the Tg^{2A} and Tg^{2B} loci were significantly easier to thresh than PI 193883 indicating that the *Q* allele had a stronger effect on conferring the free-threshing character than either tg^{2A} or tg^{2B} (Table 3.5). Lines with domesticated Rusty alleles at two of the three loci (i.e. classes $Tg^{2A}tg^{2B}Q$, $tg^{2A}Tg^{2B}Q$, and $tg^{2A}tg^{2B}q$) were easier to thresh than lines with one or no

domestication alleles, were more difficult to thresh than lines with domestication alleles at all three loci, and were not significantly different from each other (Table 3.5). Overall, these results suggest that the effects of *Q*, *tg*^{2A}, and *tg*^{2B} are mostly additive and they all contribute to the free-threshing character, with *Q* having slightly larger effects than *tg*^{2A} and *tg*^{2B}.

Table 3.5. Grouping of the threshability trait associated genotypic classes on the basis of domestication alleles (Rusty) or primitive alleles (PI 193883) on 2AS, 2BS, and 5AL QTL regions associated with free-threshing governing loci *Tg*^{2A}, *Tg*^{2B}, and *Q*, respectively

Genotype[a]	Number of RILs	Threshability	
		Mean[b]	Standard Deviation
Tg^{2A}*Tg*^{2B}*Q*	9	3.05_{b}	0.71
Tg^{2A}*tg*^{2B}*Q*	27	2.40_{c}	0.79
tg^{2A}*Tg*^{2B}*Q*	8	2.25_{c}	0.48
tg^{2A}*tg*^{2B}*Q*	23	1.31_{d}	0.31
Tg^{2A}*Tg*^{2B}*q*	11	3.81_{a}	0.19
Tg^{2A}*tg*^{2B}*q*	38	3.59_{a}	0.46
tg^{2A}*Tg*^{2B}*q*	7	3.59_{a}	0.54
tg^{2A}*tg*^{2B}*q*	31	2.47_{c}	0.66

[a]Primitive cultivated emmer alleles are shown in red and domesticated durum wheat alleles are shown in green.

[b]Means followed by different letters are significantly from each other at the 0.05 level of probability.

3.4. Discussion

3.4.1. Days to heading

DTH in wheat is under the control of three genetic regulatory systems: vernalization (*Vrn*), photoperiod (*Ppd*) and earliness per se (*Eps*) (see Kamran et al. 2014 for review). In the current study, three DTH QTL identified on the chromosomes 1A, 5A, and 7B most likely correspond to previously identified wheat heading/flowering time-influencing genes including *ELF3*, *Vrn-A1*, and *FT-B1*, respectively (Kato et al. 1999, 2000; Bullrich et al. 2002; Yan et al. 2003, 2004; Faris et al. 2014c; Kamran et al. 2014; Nitcher et al. 2014; Alvarez et al. 2016; Zou et al. 2017). The 7B QTL for DTH, which likely corresponded to the *FT-B1* gene, was season-specific, suggesting the existence of minor G × E interactions associated with these loci.

These results suggest that all the significant QTL identified in the RP883 population to be associated with DTH are under the control of previously identified and characterized genes. It is also interesting to note that, although the *Q* locus is often associated with DTH (Simons et al. 2006; Zhang et al. 2011), no effect of the gene was evident in this population suggesting that the pleiotropic effects of *Q* on heading time may be influenced by the genetic background.

3.4.2. Plant height

Two HT QTL located on chromosome arm 5AL coincided with *Vrn-A1* and *Q*, which are known to influence HT (Kato et al. 1999, 2000; Faris et al. 2003, 2005; Yan et al. 2003; Simons et al. 2006; Zhang et al. 2011; Debernardi et al. 2017; Greenwood et al. 2017; Zou et al. 2017). The chromosome 7B HT QTL may be the same QTL as one reported by Cadelen et al. (1998).

3.4.3. Spike morphology

The spike morphology traits evaluated in this research were SL, SPS, and SC. The *Q* locus was strongly associated with SL, which has been previously demonstrated as one of the major pleiotropic effects of *Q* (Muramatsu 1963, 1986; Faris and Gill 2002; Faris et al. 2003, 2005; Simons et al. 2006; Zhang et al. 2011), and the *Vrn-A1* locus (or one tightly linked) had a large effect on the number of spikelets per spike. To our knowledge, *Vrn-A1* has not been previously shown to influence SPS. Regardless, the effects of these two loci on SL and SPS resulted in both having a significant association with SC. The QTL on 1BL for SPS and the QTL for SL on chromosomes 4A and 4B had very minor effects and require further validation.

3.4.4. Rachis fragility

The brittle rachis trait was lost before the formation of cultivated emmer wheat, and it occurred due to mutations in the *Br* genes on group 3 chromosomes (Avni et al. 2017). Although the rachis in cultivated emmer is not extremely brittle, it is still rather fragile and easily

disarticulates upon application of a relatively weak mechanical force. This rachis fragility has been demonstrated to be due to the pleiotropic effects of the *q* allele in numerous studies (Leighty and Boshnakian 1921; Mackey 1954; Singh et al. 1957; Muramatsu 1979, 1985; Faris and Gill 2002; Simons et al. 2006; Zhang et al. 2011) including the recent study by Faris et al. (2014c) that employed the BP025 population. In that study, the *q* locus explained 28% of the variation in RF. On the contrary, the *q* locus was not associated with RF in the RP883 population suggesting that other genes in some genetic backgrounds may influence the effects of *q* on this trait. Indeed, early observations by Muramatsu (1979, 1985) in other accessions of cultivated emmer support this notion.

The only significant QTL found to be associated with RF was *QFra.fcu-2B*, which was located on the long arm and explained 13.6% of the variation. This QTL was significant only in the Fall 2015, and further validation is therefore needed. However, it is possible that this QTL represents a homoeoallele of the RF gene reported on chromosome arm 2AL in other biparental populations (Peng et al. 2003; Peleg et al. 2011).

3.4.5. Threshability and the evolution of free-threshing tetraploid wheat

The results of this research agree with those of Faris et al. (2014c) in that cultivated emmer wheat harbors the primitive non-free-threshing alleles at all three major threshability loci including Tg^{2A}, Tg^{2B}, and *q*. In addition to these three loci, Faris et al. (2014c) identified two additional QTL for TH with relatively minor effects. One of these minor QTL was located on chromosome 3A and the other was on chromosome 2A proximal to the Tg^{2A} locus. These two QTL explained only 2.2 and 5.4% of the variation, respectively, and were not observed in the RP883 population. In the RP883 population, the Tg^{2A}, Tg^{2B}, and *q* were the only loci associated with TH.

Although *Tg*^{2A}, *Tg*^{2B}, and *q* were associated with TH in both the BP025 (Faris et al. 2014c) and the RP883 populations, differences in the magnitudes of effects of these loci, mainly *Tg*^{2A} and *Tg*^{2B}, were observed. In the BP025 population, *Tg*^{2A} had relatively minor effect explaining only 5.7% of the variation, whereas *Tg*^{2B} had more substantial effects explaining 17.2%. The situation was essentially reversed in the RP883 population where *Tg*^{2A} explained 22.5% of the variation and *Tg*^{2B} explained only 6.7%. The *q* locus had about the same effect in both populations explaining 30.9 and 32.8% of the variation in the BP025 and RP883 populations, respectively. These results suggest that different *Tg* alleles may exist or that *Tg* genes are influenced by the genetic background.

Analysis of mean TH values of the different genotypic classes involving *Tg*^{2A}, *Tg*^{2B}, and *q* indicated that the *Q* allele had a larger effect on threshability than either of the other two loci. It is also interesting to note that neither *Tg*^{2A} nor *Tg*^{2B} are espistatic to *Q* – as is known to be the case for *Tg*^{2D} – because lines having the *Tg*^{2A}*Tg*^{2B}*Q* genotype were significantly easier to thresh (score = 3.05) than lines with *Tg*^{2A}*Tg*^{2B}*q* (score = 3.81) (Table 3.5). Instead, it appears that the effects of these three genes in this population are mostly additive, because lines with two domestication alleles were easier to thresh than lines with only one domestication allele, and lines with all three domestication alleles were easier to thresh than lines with two domestication alleles.

However, to attain a completely free-threshing phenotype, domestication alleles at all three loci are required, and the mutations that gave rise to these alleles must have occurred within a very short time period. Archaeological evidence indicates that cultivated emmer first appeared from 9,500 to 9,000 years ago in the Levant (Nesbitt and Samuel 1996), and durum appeared from 7,500 to 6,500 years ago, which means that all three genes would have undergone mutation

within a span of 1,500 to 3,000 years. However, to further complicate the picture, there is archaeological evidence of an extinct free-threshing tetraploid, known as *T. turgidum* ssp. *parvicoccum,* which shows up in the record about the same time as cultivated emmer (Kislev 1980). It is possible that durum evolved from cultivated emmer by way of ssp. *parvicoccum*. If this scenario is correct, then the triple mutation in the domestication genes would have happened even more rapidly – within about 1,000 years or possibly less.

Some researchers have questioned the existence of *T. turgidum* ssp. *parvicoccum* because few samples have been found and characterized (Nesbitt 2001). But regardless of whether or not it existed, it is apparent that the triplet of domestication mutations must have occurred very rapidly. To date, true transitional genotypes have not been identified. Muramatsu (1979) reported the presence of the *Q* allele in *T. turgidum* ssp. *dicoccum* var. *liguliforme,* and Faris et al. (unpublished) recently identified an additional *Q*-bearing cultivated emmer accession. However, subsequent sequence and phylogenetic analysis revealed that these two cultivated emmer accessions likely obtained *Q* through more recent hybridization events resulting in gene flow of the *Q* allele from a domesticated genotype (Faris et al. unpublished).

The tetraploid subspecies involved in the hybridization event with *Ae. tauschii* that led to the formation of hexaploid *T. aestivum* is still a matter of debate as well. It is generally accepted that either cultivated emmer, or a free-threshing ssp. such as *parvicoccum* or *durum* was involved (Faris 2014). In light of our findings, it is, perhaps, less likely that cultivated emmer was involved because the resulting hexaploid would have had the genotype $Tg^{2A}Tg^{2B}Tg^{2D}q$, and therefore would have had to undergo critical mutations in all four genes extremely rapidly, because no primitive or transitional forms of *T. aestivum* ssp. *aestivum* have been identified, and the archaeological and genetic evidence agree that free-threshing ssp. *aestivum* originated about

8,500 to 8,000 years ago (Nesbitt 2001; Huang et al. 2002). Under this scenario, it is possible that the free-threshing tetraploids could have obtained the mutated domestication alleles tg^{2A}, tg^{2B}, and *Q* from free-threshing hexaploid wheat through gene flow (Fig. 3.4).

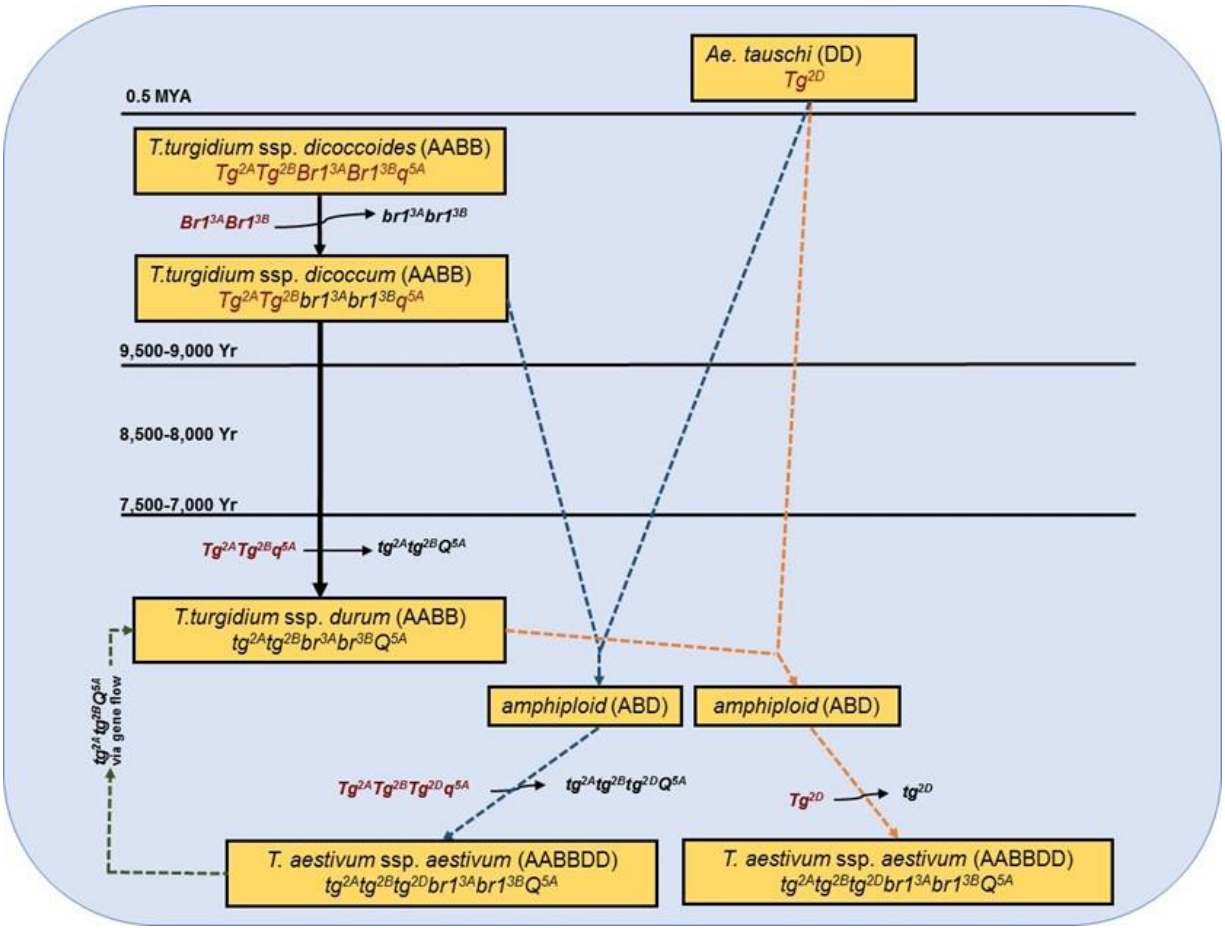

Fig. 3.4. Model representing two hypothetical scenarios for the evolution of free-threshing wheat. The pre-domestication and domesticated alleles for brittle rachis (*Br*), tenacious glume (*Tg*), and non-free-threshing (*q*) are shown in *red* and *black* font, respectively. The *orange dashed* lines represent scenario 1 in which durum wheat is the progenitor of free-threshing hexaploid wheat. The *blue dashed* lines represent scenario 2 in which cultivated emmer was involved in the formation of hexaploid wheat. In scenario 2, free-threshing tetraploid wheat (durum) would have acquired the domestication alleles tg^{2A}, tg^{2B}, and *Q* from free-threshing hexaploid wheat via gene flow (*green dashed* line).

A second scenario is that a free-threshing tetraploid with genotype $tg^{2A}tg^{2B}Q$ was involved in the amphiploidization event that gave rise to ssp. *aestivum* (Fig. 3.4). Under this scenario, the resulting hexaploid would have had to undergo a mutation in only one gene, Tg^{2D},

to become fully free-threshing. The current archaeological data would suggest that ssp. *parvicoccum*, which dates to about 9,000 years ago (Kislev 1980) would be a better candidate than ssp. *durum*, because the latter does not show up in the archaeological record until 7,500 to 6,500 years ago, or about 500 to 2,000 years after the earliest findings of free-threshing ssp. *aestivum*.

Clearly, there are many unanswered questions regarding the timing and genetic pathways leading to both tetraploid and hexaploid wheat domestication. Cloning of the *Q* gene allowed Simons et al. (2006) to show that the mutation giving rise to the domestication allele likely occurred only once, but it is unknown whether or not the mutation occurred first in a tetraploid or in a hexaploid. Perhaps the cloning of the *Tg* genes will provide further insights as to which subspecies the *tg* alleles first originated, which would help to further assemble the wheat domestication puzzle.

3.5. References

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4. MAPPING AND CHARACTERIZATION OF STEM RUST RESISTANCE GENES DERIVED FROM CULTIVATED EMMER

4.1. Introduction

Wheat (*Triticum* spp.) production has been threatened by the disease stem rust for thousands of years (Saari and Prescott 1985; Singh et al. 2015). Stem rust is caused by the biotrophic fungal pathogen *Puccinia graminis* f. sp. *tritici* Eriks & E.Henn (*Pgt*), which has an alternative host *Berberis vulgaris* (barberry*)*. Before 1928, the north central states experienced major stem rust epidemics in 1878, 1904, and 1916, due to the presence of barberry in these geographical areas (Roelfs 1978). Although barberry was eradicated from most of the region, the overwintering of *Pgt* in the Southern states led to major epidemics in the 1930's and 1950 (Roelfs 1978). After 1950, breeding for resistance became a major priority to combat losses caused by stem rust. Collectively, eradication of the alternative host barberry and resistance breeding reduced the threat of stem rust epidemics until 1998 (Singh et al. 2008; Simons et al. 2011).

In 1998, the stem rust race TTKSK, commonly known as Ug99, caused a major epidemic in Uganda by overcoming the frequently used resistance gene *Sr31* (Pretorius et al. 2000; Jin and Singh 2006; Fetch 2007; Singh et al. 2008; Lopez-Vera et al. 2014). The majority of wheat germplasm and cultivars currently grown are susceptible to this pathogen, making it a major concern for the wheat community. *Pgt* is known for having high mutation, recombination and migration rates, and within two decades thirteen variants (TTKSK, TTKSF, TTKST, TTTSK, TTKSP, PTKSK, PTKST, TTKSF+, TTKTT, TTKTK, TTHSK, PTKTK, and TTHST) of the Ug99 lineage have been detected in multiple African countries (http://rusttracker.cimmyt.org; Guerrero-Chavez et al. 2015; Fetch et al. 2016).

In addition to the Ug99 group, additional *Pgt* races including TRTTF, TTTTF, and TKTTF have been detected in the last decade (Jin 2005; Olivera et al. 2012, 2015, and 2017). Among these, TRTTF was detected in Yemen in 2006 and found to be virulent against *Sr36*, *SrTmp*, *Sr1RS^{Amigo}*, and *Sr9e* (Olivera et al. 2012). Stem rust resistance genes *Sr9e* and *Sr13* are frequently deployed in North American durum (*T. turgidium* ssp. *durum* L., 2*n* = 4*x* = 28, AABB*)* cultivars (Olivera et al. 2012; Zhang et al. 2017). Therefore, TRTTF virulence against *Sr9e* raises concerns for durum wheat production (Olivera et al. 2012). Durum wheat is widely used in pasta and bread production. North Dakota is the major producer of durum in the United States (http://www.ndwheat.com). In addition, other states and Canadian provinces (South Dakota, North Dakota, Minnesota, Manitoba and Saskatchewan) are also known for wheat production. This whole wheat-producing North America region has experienced multiple stem rust outbreaks since the 1900's. Therefore, the possibility of future stem rust outbreaks cannot be ruled out (Roelfs 1978). Hence, there is a need to develop new resistant wheat varieties with enhanced genetic diversity in order to eliminate these regional outbreaks.

To enhance genetic diversity in wheat varieties, the primary, secondary, and tertiary gene pools have been frequently exploited. Secondary and tertiary wheat gene pools provide rich sources of disease resistance genes. However, these genes are almost always associated with linkage drag effects, which can ultimately lead to agronomic losses. At present, 39 *Sr* genes are known to be effective against the Ug99 lineage (Singh et al. 2015). Of these, three TTKSK resistance genes (*Sr2*, *Sr13*, and *Sr14*) are derived from cultivated emmer (*T. turgidium* ssp. *dicoccum* (Schrank) Schübl, 2*n* = 4*x* = 28, AABB), which is a member of the wheat primary gene pool (Singh et al. 2015). Besides these, six other TTKSK-ineffective genes (*Sr9d*, *Sr9e*, *Sr9g*, *Sr11*, *Sr12*, and *Sr17*) were derived from cultivated emmer as well (Singh et al. 2015).

Sr2 is a non-race specific, slow rusting, adult-plant resistant gene, that has been used for almost a hundred years as a durable source of resistance against stem rust (Singh et al. 2008; Mago et al. 2014). *Sr13* and *Sr14* are not promising for future stem rust preventions due to their race specificity and ineffectiveness under high disease pressure (Singh et al. 2015). Although *Sr13* is a good source of resistance in durum cultivars, its temperature-effected functionality and ineffectiveness in Ethiopia and India (Periyannan et al. 2014) make it unsuitable in the long term. The futility of existing *Sr* genes to control the disease for a long-time period, along with high mutation rates and recombination frequencies of *Pgt* makes it essential that wheat researchers continue to identify, characterize, and deploy new sources of resistance.

The first objective of this study was to determine the number of genes conferring stem rust resistance in the *T. turgidum* ssp. *dicoccum* accession PI 193883. The second objective was to determine the chromosome location of the resistance genes. The third objective was to develop molecular markers suitable for marker-assisted selection (MAS) of these resistance genes.

4.2. Material and Methods

4.2.1. Plant materials

The *T. turgidum* ssp. *dicoccum* accession PI 193883 was previously reported to carry seedling stem rust resistance against *Pgt* races TTKSK, TRTTF, TTTTF, TPMKC, RKQQC, QTHJC, and MCCFC (Olivera et al. 2012). The tetraploid durum wheat line Rusty is nearly universally susceptible to *Pgt* races. A population of 190 recombinant inbred lines (RILs) (F_7 generation) derived from a cross between Rusty and PI 193883 (designated as the RP883 population) was previously evaluated for agronomic and domestication traits (Chapter 3). In the current chapter, the same population was screened with different *Pgt* races.

4.2.2. Stem rust screening

Stem rust screenings were conducted with *Pgt* races TMLKC, TTKSK, and TRTTF. Screening with the local North America race TMLKC was done at the USDA-ARS Cereals Crops Research Unit, Fargo, North Dakota in two replications (2015 and 2016). Stem rust screening with *Pgt* races TTKSK and TRTTF was done at the USDA-ARS Cereal Disease Laboratory, St. Paul, MN in three replications, including two replications in a growth chamber (2015) and one replication in a greenhouse (2016). Stem rust evaluations were done on the parents (Rusty and PI 193883), F_1 plants, F_2 plants, and the RP883 population.

The screenings at Fargo with TMLKC were conducted using the methods described by Williams et al. (1992). Seeds were planted in cones (3.8 cm in diameter and 21 cm in length) in a greenhouse and were allowed to germinate for 6-8 days. A heat shock treatment was given to 80°C long-term stored urediniospores by incubating glass vials containing the spores at 45°C for 7-10 minutes. After the shock treatment, the spores were laid into '00 gelatin capsules.' The gelatin capsules were placed in the rehydration chamber containing ammonium sulphate (KOH) solution for 3-4 hours. The hydrated urediniospores were suspended in light mineral oil 'Soltrol 170.' Plants were inoculated by spraying the oil suspended urediniospores, and the plants were allowed to air dry for 20 minutes. After inoculation, plants were placed in mist chambers with 100% relative humidity in the dark for 20-22 hours at 18°C. The misters were turned off after 20-22 hours and fluorescent lights were turned on. The plants were allowed to dry under the fluorescent lights for 3-4 hr. Then plants were removed from the mist chambers and transferred to the greenhouse at 18 ± 2°C with a photoperiod of 16 hr.

The stem rust evaluation for *Pgt* races TTKSK and TRTTF was done based on the method described by Rouse et al. (2011). Infection types (IT) were recorded 12-14 days after

inoculation by using the Stakman et al. (1962) scoring pattern with five basic reaction types "0, ;, 1, 2, 3, 4" and additional symbols of "+" and "–" represent the size variation of pustules (Roelfs and Martens 1988). In order to map the chromosome 2BL QTL as a Mendelian trait, TTKSK and TRTTF IT scores for the RP883 population were converted to resistance and susceptible categories. RILs with IT scores of 0 to 2 were considered resistant and ITs of 3-4 were classified as susceptible. To check the homogeneity of variances between two replications of F_2 plants Bartlett's test (Snedecor and Cochran 1989) with PROC GLM in the SAS program version 9.3 (SAS institute 2011) was used. For QTL analysis, each RIL IT score of individual *Pgt* races was linearized and rounded into a 0 to 9 scale by using the method described by Zhang et al. (2014), where 0 was immune and 9 was higly susceptable.

4.2.3. Marker development and validation

The RP883 population linkage maps comprised of 9281 SNPs and 65 simple sequence repeat (SSR) markers were previously reported in Chapter 3. The same maps were used to determine the *Sr* genes governing resistance against *Pgt* races, TTKSK, TRTTF, and TMLKC. In addition, new SNP-based semi-thermal asymmetric reverse PCR (STARP) and International Wheat Genome Sequencing Consortium (IWGSC) scaffold-based SSR markers were also developed for stem rust resistance QTL regions on chromosome arms 2BL and 6AL. To develop SSR markers for the 2B QTL region, BLASTn (Altschul et al. 1997) against wheat 2BL survey sequences (http://wheat-urgi.versailles.inra.fr) was used on sequences flanking SNPs mapped to the 2BL region. The scaffold spanning the flanking SNPs of the 2BL QTL region was selected for marker development. The Batchprimer3 (You et al. 2008) and WebSat (Martins et al. 2009) software programs were used to develop SSRs from the selected scaffold. For the development of allele-specific STARP primers based on the method developed by Long et al. (2017), SNPs

mapped in QTL regions on chromosome 2B and 6A were selected. Contextual sequences of SNPs that flanked the QTL regions were subjected to BLASTn searches against the wheat genome scaffolds (http://wheat-urgi.versailles.inra.fr). SNP sequences that were specific to chromosome 2B were further selected for marker development. Sequences that immediately flanked the SNP contextual sequences were used to design the allele-specific forward and reverse primers as described in Long et al. (2017).

For SSR marker evaluation, polymerase chain reaction (PCR) was applied based on the Röder et al (1998) protocol with the following conditions: initial denaturation at 94°C for 5 minutes, 35 cycles with 0.5 minutes at 94°C, annealing for 0.5 minutes at 61°-54°C with -0.2°C at each cycle, 1.5 minutes for extension at 72°C, and final extension at 72°C for 7 minutes. Amplified fragments were separated on 6% non-denaturing polyacrylamide gels, stained with GelRed™ nucleic acid stain, (Biotium Corporate, Hayward, CA) and scanned using a Typhoon FLA 9500 variable mode imager (GE healthcare Biosciences, Waukesha, WI).

STARP markers were evaluated according to the method described by the Long et al. (2017). Five primers were used in a PCR reaction; two allele specific primers (Forward 1 and 2), a common reverse primer, and two universal priming element-adjustable (PEA) primers. For gel-based analysis of STARP markers, 33 cycles were used, however 46 cycles were used for the gel-free marker analysis with the CFX384 Touch^{TM} Real-Time PCR detection system (Bio-Rad Laboratories, Inc., Foster City, CA, USA). The amplified fragments were separated and visualized as described for SSR markers. The STARP marker developed for the chromosome 6AL QTL region was used in two other *Sr* gene mapping studies in Dr. Steven S. Xu's lab, hence it was given a different desiginated than the other STARP markers developed in the current study. To validate the utility of newly developed SSR and STARP markers for MAS breeding, a

validation panel consisting of 48 durum and common wheat cultivars from the U.S., Canada, China, Australia, Brazil, and Italy was used. In addition, Chinese Spring (CS) and CS homoeologous nullisomic-tetrasomic lines for the chromosome group 6 (N6AT6B, N6BT6A, and N6DT6B) were used to assign fragments to specific chromosomes.

4.2.4. Linkage and trait analysis

The newly developed markers were mapped in the RP883 population on linkage groups 2B and 6A using the program MapDisto 1.8.2.1 (Lorieux 2012) and the Kosambi mapping function (Kosambi 1943). To get the best order of markers in a linkage group, the 'order sequence,' 'check inversions,' 'ripple order,' and 'drop locus' commands were used. The program QGENE (4.4.0) (Joehanes and Nelson 2008) was used to determine the genomic region associated with phenotypic variation in the RP883 population using single-trait multiple interval mapping (MIM). A permutation test was performed with 1000 iterations to determine the critical LOD threshold (3.5) for declaring significance of a QTL. The amount of phenotypic variation explained by an individual QTL was determined by calculating the coefficient of determination (R^2).

4.2.5. Grouping of RILs based on chromosomes 2BL and 6AL QTL region alleles

The 190 RILs were classified into four groups based on their allelic states for the two stem rust resistance conferring QTL on chromosome arms 2BL and 6AL, where PI 193883 and Rusty alleles conferred resistance or susceptibility, respectively. The flanking markers, *IWB56465-IWB2143* and *IWB34398-Xbarc104* were used for making selection in *QSr.fcu-2B* and *QSr.fcu-6A* regions, respectively. Both QTL regions were used to select the RILs containing alleles of a single QTL (*QSr.fcu-2B* or *QSr.fcu-6A*), both QTL, and no QTL (Rusty alleles). The

mean IT score of each genotypic class was used to determine the Fisher least significance difference (LSD) at $\alpha = 0.05$.

4.3. Results

4.3.1. Stem rust reaction

Stem rust evaluations indicated that Rusty was susceptible to *Pgt* races TTKSK, TRTTF, and TMLKC with an average IT score of 3+ (Table 4.1). On the other hand, PI 193883 was resistant to TTKSK (IT = 2), TRTTF (IT = 2/22-), and TMLKC (IT = 2) (Table 4.1). Stem rust inoculations on F_1 plants displayed different IT scores for *Pgt* races TTKSK, TRTTF, and TMLKC. TTKSK and TRTTF had average IT scores of 3/33+ and 2+3/32+, respectively (Table 4.1). However, F_1 plants were resistant to the *Pgt* race TMLKC with an IT of 2 (Table 4.1). Stem rust evaluation with TTKSK showed 33 F_2 plants with IT range from 2 to 2+ and 101 F_2 plants with IT = 3+. On the other hand, for TRTTF, it was difficult to differentiate between the resistant class (IT = 2- to 2+3) and the susceptible class (IT = 3). The F_3 plant stem rust screening for race TRTTF showed that lots of plants were segregating with variable IT range, hence it was not possible to classify the F_2 plants in to a specific gene ratio. With TMLKC, out of 197 plants, 125 were susceptible (IT =4/3) and the remaining 72 plants were resistant (IT = 2- to 32). Stem rust screening of the RP883 population showed IT scores between 2 and 3+ for TTKSK, TRTTF, and TMLKC (Fig 4.1).

Table 4.1. Stem rust reaction for parents, Rusty and *T. turgidum* ssp. *dicoccum* accession PI 193883, F_1 plants for three *Pgt* races TTKSK, TRTTF, and TMLKC inoculations

Race	Parents		F_1 Plant
	Rusty	PI 193883	
TTKSK	3+	2	3/33+
TRTTF	3+	2/22-	2+3/32+
TMLKC	3+	2	2

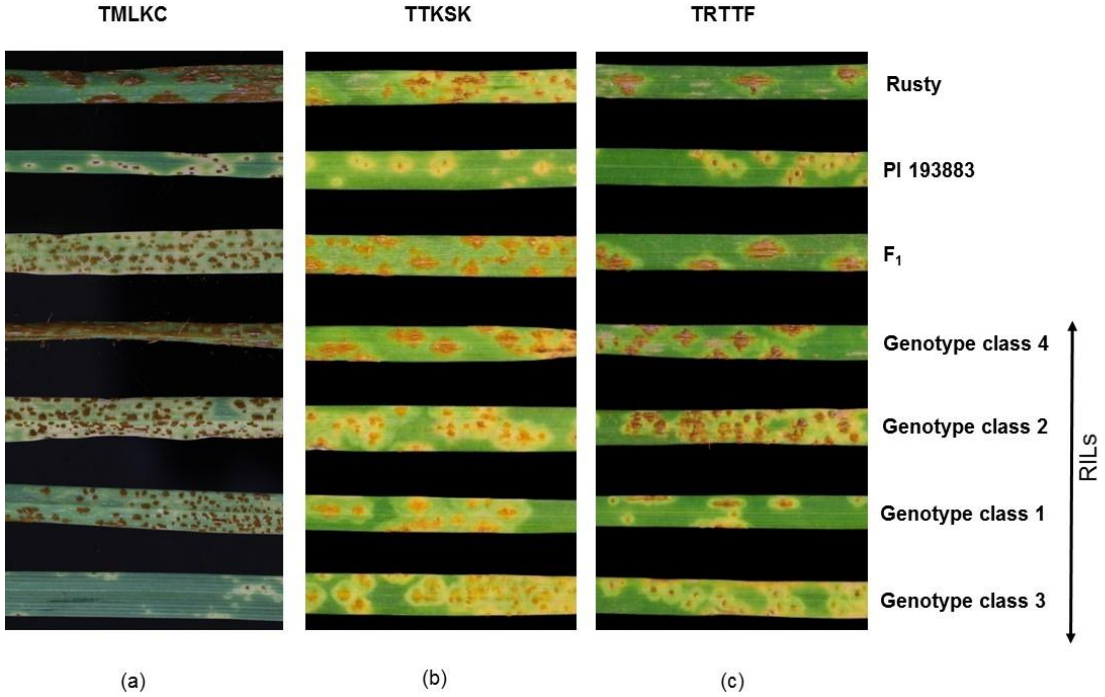

Fig 4.1. Stem rust inoculations of *Pgt* races TMLKC, TTKSK, and TRTTF on the parents, Rusty and *T. turgidum* ssp. *dicoccum* accession PI 193883, and four selected RILs. Genotype classes 1, 2, 3, and 4 represent RILs carrying only *QSr.fcu-2B*, only *QSr.fcu-6A*, both *QSr.fcu-2B* and *QSr.fcu-6A*, and neither *QSr.fcu-2B* or *QSr.fcu-6A*, respectively.

4.3.2. QTL analysis

Two major QTL were identified for resistance against *Pgt* races TTKSK, TRTTF, and TMLKC, and were located on chromosome arms 2BL and 6AL. The QTL on chromosome 2BL was present at position 78 cM, flanked by SNPs *IWB56465* and *IWB55767*, and designated *QSr.fcu-2B*. The *QSr.fcu-2B* LOD values for *Pgt* races TTKSK, TRTTF, and TMLKC were 31.1,

63.0, and 21.2, respectively (Fig. 4.2, Table 4.2). The level of variation explained by the *QSr.fcu-2B* region was different among the *Pgt* races, explaining 63.6 and 83.5% of the varation for *Pgt* races TTKSK and TRTTF, respectively. However, it explained only 27.4% of the varation in stem rust caused by TMLKC (Fig. 4.2, Table 4.2). Among the 190 RILs, three showed inconsistency in the stem rust reaction type among the *Pgt* races TRTTF and TTKSK. The IT score of RILs for the TTKSK race was more clear and easy to distinguish between resistant and susceptible lines (as mentioned in TRTTF analysis for F_2 plants). Therefore, the TTKSK IT score was used for the mapping the PI 193883 derived resistance as a Mendelian trait, and the gene was temporarily designated as *Sr883*. *Sr883* was narrowed to a region spanning 0.6 cM between the SNP markers *IWB56465* and *IWB55767* on chromosome arm 2BL (Fig. 4.2).

Another major QTL associated with resistance against *Pgt* races TMLKC, TTKSK, and TRTTF was present on chromosome arm 6AL between markers *IWB3057* and *Xbarc104*, and was designated *QSr.fcu-6A* (Fig. 4.3). The LOD values for *QSr.fcu-6A* were 3.8, 7.4 and 26.9 for TTKSK, TRTTF and TMLKC, respectively (Fig. 4.3, Table 4.2). This region explained 35.4% of the variation in stem rust caused by TMLKC, and approximately 5.0 and 5.3% of the variation for TTKSK and TRTTF, respectively (Table 4.2).

4.3.3. Marker development and physical mapping of the *QSr.fcu-2B* region

For the *QSr.fcu-2B* region, two STARP markers (*Xfcp716* and *Xfcp717*) developed from flanking SNPs and three SSR markers (*Xfcp718*, *Xfcp719*, and *Xfcp720*) developed from the IWGSC scaffold 57495 all co-segregated with the *Sr883* locus (Table 4.3, 4.4, Fig. 4.2). *Xfcp716* and *Xfcp717* were both dominant markers with the former in coupling with *Sr883* and the latter in repulsion. Among the three *QSr.fcu-2B*-associated SSR markers, *Xfcp719* and *Xfcp720* were co-dominant and *Xfcp718* was dominant and in coupling with *Sr883*. One co-dominant STARP

marker (*Xrwgsnp7*) was developed from the SNP *IWB34398* mapped in the *QSr.fcu-6A* region (Table 4.3, Fig. 4.3, 4.4). Among the three STARP markers developed in the current study, only *Xrwgsnp7* worked efficiently with the gel-free system. *Xfcp716* amplified the PI 193883 fragment in common wheat lines from China, Canada, and Brazil (Table 4.5). However *Xfcp717* was able to differentiate between all wheat cultivars and lines in the validation panel. *Xrwgsnp7* was able to differentiate Rusty and PI 193883 alleles in durum cultivars, but in common wheat lines fragments associated with both parental alleles were amplified (Table 4.5). On the basis of homoeologous chromosome group 6 nullisomic-tetrasomic analysis, only Rusty-associated alleles were specific to chromosome 6A, but the *Xrwgsnp7* marker amplified homologous copies on other genomes of group 6 (Fig. 4.4). From three SSR markers, the dominant *Xfcp718* marker worked in US based germplasm, whereas the co-dominant markers *Xfcp719* and *Xfcp720* were able to differentiate the RP883 parental alleles across the validation panel (Table 4.5). The physical distance between SNP and SSR sequences of *QSr.fcu-2B* region containing the *Sr883* gene was 27.2 Mb using the IWGSC RefSeq v1.0 (Table 4.6, Fig. 4.2).

4.3.4. Comparison of the *Sr883* region with other *Sr* genes mapped on chromosome arm 2BL

Four stem rust resistance genes have been identified on chromosome arm 2BL: *Sr9*, *Sr16*, *Sr47*, and *Sr28* (Singh et al. 2015). Of these four, *Sr28* and *Sr47* confer resistance to the Ug99-group (Singh et al. 2015). Besides these, Zurn et al. (2014) reported the mapping of *T. dicoccum* PI 626573-derived *SrWLR* in the *Sr9* region of chromosome arm 2BL. Comparisons between previously reported *Sr* genes on chromosome arm 2BL and *Sr883* using SSRs as anchoring markers revealed that TTKSK resistance genes *Sr9h* and *SrWLR* were mapped distal to SSR

marker *Xgwm388*, while the *Sr883* gene mapped proximal to SSR *Xgwm388* (Fig. 4.5) (Rouse et al. 2014; Zurn et al. 2014).

4.3.5. Stem rust reaction analysis for genotypic classes

Based on allelic states for the two QTL regions, the RP883 population was classified into four different genotypic classes where classes 1, 2, 3, and 4 represent RILs carrying only *QSr.fcu-2B,* only *QSr.fcu-6A*, both *QSr.fcu-2B* and *QSr.fcu-6A*, and neither QTL, respectively (Table 4.7). The LSD analysis clustered these four genotypic classes into three groups with significantly different IT scores for *Pgt* races TTKSK, TRTTF and TMLKC. (Table 4.7). RILs with only *QSr.fcu-2B* or both QTL had the same level of resistance (IT = 5.5/5.6) against TTKSK and TRTTF (Table 4.7). However, RILs with only *QSr.fcu-6A* (mean IT = 8.1) or neither QTL (mean IT = 9.0) were not effective against these two races. Interestingly, for TMLKC, RILs with only *QSr.fcu-2B* or only *QSr.fcu-6A* were equally effective, wheras RILs with both QTL showed high levels of resistance (IT = 4.3) (Table 4.7). RILs with neither QTL were highly susceptible (IT = ~9.0).

Table 4.2. QTLs associated with resistance against *Pgt* races TMLKC, TTKSK, and TRTTF, their LOD values, coefficient of determination (R^2) and additive values

Pgt race	QTL	Chr[a]	Position (cM)	LOD	$R^2 \times 100$	Marker interval	Additive effects[b]
TMLKC	*QSr.fcu-2B*	2BL	78	21.2	27.4	*IWB56465-IWB2143*	-1.0
	QSr.fcu-6A	6AL	138	26.9	35.4	*IWB34398-Xbarc104*	-1.2
TTKSK	*QSr.fcu-2B*	2BL	78	31.1	63.6	*IWB56465-IWB2143*	-1.1
	QSr.fcu-6A	6AL	136	3.8	5.0	*IWB3057-Xbarc104*	-0.3
TRTTF	*QSr.fcu-2B*	2BL	78	63.0	83.5	*IWB56465-IWB2143*	-1.5
	QSr.fcu-6A	6AL	136	7.4	5.3	*IWB3057-Xbarc104*	-0.4

[a]Chr.- Chromosomal arm

[b]Additive effects: Negative values indicate the value derived from cultivated emmer accession PI 193883.

Table 4.3. The semi-thermal asymmetric reverse PCR (STARP) marker, their source SNP ID, forward and reverse primers sequences, approximate product size and their inheritance

Marker	Chr.[a]	Source SNP ID	Primer			
			Type[b]	Sequence (5'-3')[c]	~ Product Size (bp)	Inheritance/phase
Xfcp716	2BL	*IWA6016*	F1	{tail-2}CGTTCCGCTTTTAAAGCA	25, 50	Dominant/ Coupling
			F2	{tail-1}CGTTCCGCTTTTAAGACG		
			R	GCTACCACCATGTACGATTAATAGG		
Xfcp717	2BL	*IWB39516*	F1	{tail-1}GGCTCGATTGCCTCGT	25, 50	Dominant/ Repulsion
			F2	{tail-2}GGCTCGATTGCCCTGC		
			R	CAAAACCGTGATCTCCACAAAA		
Xrwgsnp7	6AL	*IWB34398*	F1	{tail-1}AGCACACTACTACGAGACAAG	105, 110, 125, 135	Co-dominant
			F2	{tail-2}AGCACACTACTACGAGAACAT		
			R	CGACCCATACTCAAGACCATCTG		

[a]Chr.- Chromosomal arm

[b]Primer type F1, F2, and R stand for Forward 1, Forward 2, and Reverse, respectively

[c]Tail-1 and 2 universal sequences are 5'-GCAACAGGAACCAGCTATGAC-3'and 5'-GACGCAAGTGAGCAGTATGAC-3', respectively.

Table 4.4. International Wheat Genome Sequencing Consortium (IWGSC) scaffold based simple sequence repeat (SSR) marker sequences, product size and inheritance

Marker	Chr.[a]	IWGSC Ref sequence coordinates	Primer			
			Type	Sequence(5'-3')	~ Product Size (bp)	Inheritance/phase
Xfcp718	2BL	452810513 - 452810662	Forward	CCCATCAGATTAACAGCTCTA	170, 250, 275	Dominant/ Coupling
			Reverse	ATGAGCAGGATAAAGACACTG		
Xfcp719	2BL	450922200 - 450922309	Forward	GCACAACTAGCGAGTGTCTAT	190, 180, 178, 160	Co-dominant
			Reverse	TATCGATTGGATTCTCTCTCA		
Xfcp720	2BL	452703134 - 452703487	Forward	TCCTCTCCTCTTCTGGGAAATC	370, 375	Co-dominant
			Reverse	CATGCGAACTAGAAAGACCCC		

[a]Chr.- Chromosomal arm

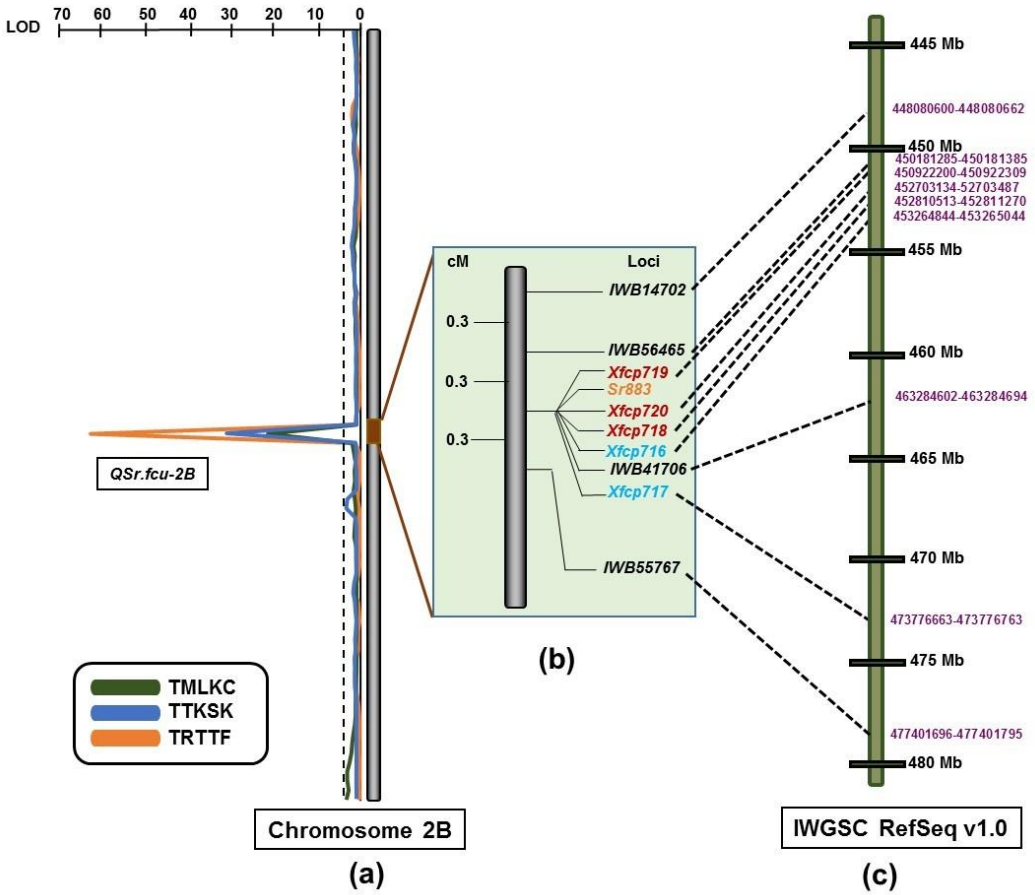

Fig 4.2. Genetic map of chromosome 2B (a) Single-trait multiple interval mapping (MIM) for three *Pgt* races TMLKC, TTKSK, and TRTTF. The dashed line represent the LOD threshold 3.5. (b) Chromosome 2B linkage map segment representing the co-segregating single nucleotide polymorphism (SNP), semi-thermal asymmetric reverse PCR (STARP) markers and simple sequence repeat (SSR) markers with the *Sr883* gene, SSR markers are represented with *red font*, STRAP markers are the light *blue font*, and *Sr883* in *orange font*. (c) The physical map of the *Sr883* region using the International Wheat Genome Sequencing Consortium (IWGSC) RefSeq v1.0, IWGSC_Ref Seq v1_position_coordinates (bp) are represented with *purple font*.

Table 4.5. Validation of the newly developed semi-thermal asymmetric reverse PCR (STARP) and simple sequence repeat (SSR) markers using durum and common wheat varieties and lines

Variety/Line	Type	Habit	Origin[a]	Amplicon (bp)[b] from markers					
				Xfcp716	*Xfcp717*	*Xrwgsnp7*	*Xfcp718*	*Xfcp719*	*Xfcp720*
Rusty	Durum wheat	Spring	ND, USA	-[c]	25, 50	110, 135	-	160, 180	370
PI 193883	Cultivated emmer	Spring	Ethiopia, Shewa	35, 75	-	105,125	170, 250, 275	178, 190	375
Strongfield	Durum wheat	Spring	Canada	-	25, 50	105,125	-	158, 176	371
Transcend	Durum wheat	Spring	Canada	-	25, 50	105,125	-	158, 176	371
Cappelli	Durum wheat	Spring	Italy	-	25, 50	110, 135	-	158, 176	371
Svevo	Durum wheat	Spring	Italy	-	25, 50	105,125	-	158, 176	371
15FAR344-6(255)	Durum wheat	Spring	ND, USA	-	25, 50	105,125	-	155, 174	371
D09557	Durum wheat	Spring	ND, USA	-	25, 50	105,125	-	158, 176	371
D09690	Durum wheat	Spring	ND, USA	-	25, 50	105,125	-	158, 176	371
D101073	Durum wheat	Spring	ND, USA	-	25, 50	105,125	-	158, 176	371
Alkabo	Durum wheat	Spring	ND, USA	-	25, 50	105,125	-	158, 176	371
Carpio	Durum wheat	Spring	ND, USA	-	25, 50	105,125	-	158, 176	371
Divide	Durum wheat	Spring	ND, USA	-	25, 50	105,125	-	158, 176	371
Grenora	Durum wheat	Spring	ND, USA	-	25, 50	105,125	-	158, 176	371
Joppa	Durum wheat	Spring	ND, USA	-	25, 50	105,125	-	158, 176	371
Langdon	Durum wheat	Spring	ND, USA	-	25, 50	105,125	-	158, 176	371
Lebsock	Durum wheat	Spring	ND, USA	-	25, 50	105,125	-	155, 174	371
Line E	Common wheat	Spring	Australia	-	25, 50	105,125	-	150, 170	370
BR34	Common wheat	Spring	Brazil	35, 75	25, 50	105, 110, 125, 135	170, 260, 275	165, 180	375
LMPG-6	Common wheat	Spring	Canada	35, 75	25, 50	105, 125	170, 260, 275	165, 180	374
Chinese Spring	Common wheat	Spring	China	35, 75	25, 50	105, 110, 125, 135	170, 260, 275	165, 180	372, 380, 400
Jimai 22	Common wheat	Winter	China	35, 75	25, 50	105, 110, 125, 135	170, 260, 275	165, 180	375
Jinqiang 5	Common wheat	Spring	China	-	25, 50	105, 110, 125, 135	-	145, 155	370
Sumai 3	Common wheat	Spring	China	35, 75	25, 50	105, 110, 125, 135	170, 260, 275	165, 180	375
Yangmai 16	Common wheat	Spring	China		25, 50	105, 110, 125, 135	170, 260, 275	-	375
Zhengmai 9023	Common wheat	Facultative	China	35, 75	25, 50	105, 110, 125, 135	170, 260, 275	165, 180	375
Zhoumai 27	Common wheat	Winter	China	35, 75	25, 50	105, 110, 125, 1355	170, 260, 275	-	374
Alsen	Common wheat	Spring	ND, USA	-	25, 50	105, 110, 125, 135	-	145, 155	370
Barlow	Common wheat	Spring	ND, USA	-	25, 50	105, 110, 125, 135	-	145, 155	370
Elgin-ND	Common wheat	Spring	ND, USA	-	25, 50	105, 110, 125, 135	-	145, 155	370
Faller	Common wheat	Spring	ND, USA	-	25, 50	105, 125	-	145, 155	370
Glenn	Common wheat	Spring	ND, USA	-	25, 50	105, 110, 125, 135	-	145, 155	370
Grandin	Common wheat	Spring	ND, USA	-	25, 50	105, 125	-	165, 180	370
ND830	Common wheat	Spring	ND, USA	-	25, 50	105, 110, 125, 135	-	145, 155	370

Table 4.5. Validation of the newly developed semi-thermal asymmetric reverse PCR (STARP) and simple sequence repeat (SSR) markers using durum and common wheat varieties and lines (continued)

Variety/Line	Type	Habit	Origin[a]	Amplicon (bp)[b] from markers					
				Xfcp716	*Xfcp717*	*Xrwgsnp7*	*Xfcp718*	*Xfcp719*	*Xfcp720*
ND833	Common wheat	Spring	ND, USA	-	25, 50	105, 110, 125, 135	-	145, 155	370
NDHRS16-12-19	Common wheat	Spring	ND, USA	-	25, 50	105, 110, 125, 135	-	145, 155	370
Reeder	Common wheat	Spring	ND, USA	-	25, 50	105, 110, 125, 135	-	145, 155	370
Steele-ND	Common wheat	Spring	ND, USA	-	25, 50	105, 110, 125, 135	-	145, 155	370
VitPro-ND	Common wheat	Spring	ND, USA	-	25, 50	105, 110, 125, 135	-	145, 155	370
IL06-14262	Common wheat	Winter	IL, USA	-	25, 50	105, 110, 125, 135	-	165, 185	350, 380
Newton	Common wheat	Winter	KS, USA	-	25, 50	105, 110, 125, 135	170, 260, 275	165, 185	350, 380
Ada	Common wheat	Spring	MN, USA	-	25, 50	105, 110, 125, 135	-	145, 155	370
Bolles	Common wheat	Spring	MN, USA	-	25, 50	105, 110, 125, 135	-	165, 180	370
Linkert	Common wheat	Spring	MN, USA	-	25, 50	105, 110, 125, 135	-	145, 155	370
Tom	Common wheat	Spring	MN, USA	-	25, 50	105, 125	-	145, 155	370
Brick	Common wheat	Spring	SD, USA	-	25, 50	105, 110, 125, 135	-	-	370
Granger	Common wheat	Spring	SD, USA	-	25, 50	105, 110, 125, 135	-	145, 155	370
Parshall	Common wheat	Spring	SD, USA	-	25, 50	105, 110, 125, 135	-	158, 175	370
Russ	Common wheat	Spring	SD, USA	-	25, 50	105, 110, 125, 135	-	150, 170	370

[a]Origin: CO, Colorado; KS, Kansas; ND, North Dakota; SD, South Dakota; MN, Minnesota; IL, Illinois; ID, Idaho
[b]Amplicon size of markers for different wheat varieties and lines in base pairs (bp)

Table 4.6. International Wheat Genome Sequencing Consortium (IWGSC) RefSeq v1.0 coordinates for the *Sr883* flanking chromosome 2B linkage map region

90K_Index/ SSR/ STARP	SNP_ID/ SSR	SNP Name	RP883_2B_map position (cM)	IWGSC_Ref Seq v1_position_coordinates (bp)
75921	*IWA838*	wsnp_CAP11_rep_c4105_1941066	77.5	448080482-448080555
75920	*IWA837*	wsnp_CAP11_rep_c4105_1940985	77.5	448080482-448080636
14702	*IWB14702*	CAP8_c3701_331	77.5	448080600-448080662
56465	*IWB56465*	RAC875_c3039_101	77.8	450181285-450181385
77429	*IWA2972*	wsnp_Ex_c25438_34703568	77.8	450184171-450184371
42378	*IWB42378*	Kukri_c20819_497	77.8	450184221-450184321
Xfcp719			78.0	450922200-450922309
Xfcp720			78.0	452703134-452703487
Xfcp718			78.0	452810513-452810662
9833	*IWB9833*	BS00066545_51	78.0	452811170-452811270
19619	*IWB19619*	Ex_c10478_746	78.0	453028409-453028509
76099	*IWA1215*	wsnp_CAP8_c775_527730	78.0	453031289- 453031489
75878	*IWA772*	wsnp_CAP11_c5255_2442548	78.0	453031560-453031759
62579	*IWB62579*	RAC875_rep_c69546_73	78.0	453031632-453031732
69314	*IWB69314*	Tdurum_contig27794_582	78.0	453078727-453078809
79774/ *Xfcp716*	*IWA6016*	wsnp_JD_c352_546108	78.0	453264844-453265044
75746	*IWA586*	wsnp_BG608232B_Ta_2_1	78.0	453267297-453267417
48056	*IWB48056*	Kukri_c8595_834	78.0	453751792-453751892
76434	*IWA1661*	wsnp_Ex_c12634_20096724	78.0	453752011-453752154
48057	*IWB48057*	Kukri_c8595_953	78.0	453752011-453752104
50814	*IWB50814*	Ra_c106383_270	78.0	453812222-453812296
51413	*IWB51413*	Ra_c21042_617	78.0	456025480-456025580
4321	*IWB4321*	BobWhite_c7326_70	78.0	456172645-456172745
39200	*IWB39200*	Ku_c33341_260	78.0	456341560-456341660
2507	*IWB2507*	BobWhite_c31986_324	78.0	457684322-457684422
79922	*IWA6240*	wsnp_JD_c9810_10594505	78.0	458244653-458244807
2143	*IWB2143*	BobWhite_c27816_523	78.0	459575922-459576022
78648	*IWA4541*	wsnp_Ex_c65790_64067038	78.0	462887158-462887358
41706	*IWB41706*	Kukri_c17062_618	78.0	463284602-463284694
39516/ *Xfcp717*	*IWB39516*	Ku_c4813_494	78.0	473776663-473776763
55767	*IWB55767*	RAC875_c25277_324	78.3	477401696-477401795
1518	*IWB1518*	BobWhite_c21705_196	81.5	494374238-494374303
73000	*IWB73000*	Tdurum_contig64751_231	81.5	494375263-494375363
1520	*IWB1520*	BobWhite_c21705_722	81.5	494375470-494375570
72999	*IWB72999*	Tdurum_contig64751_120	81.5	494375470-494375570

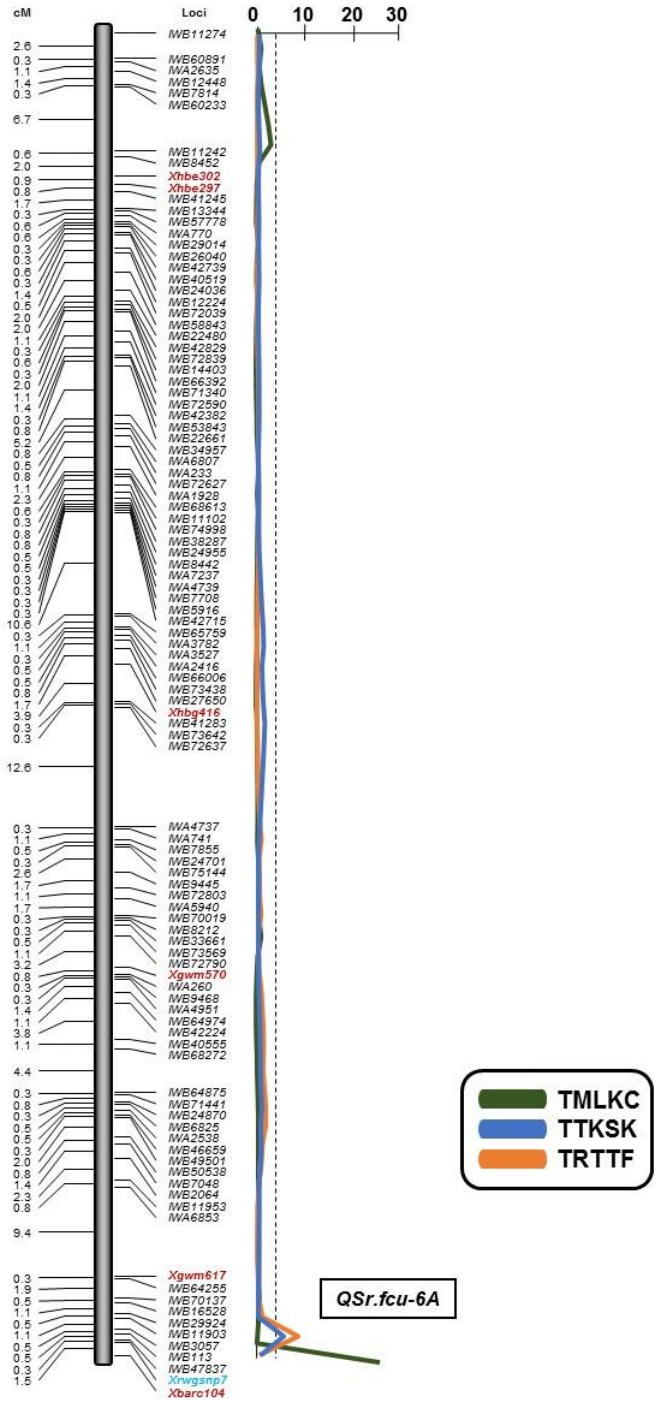

Fig 4.3. Genetic linkage map of chromosome 6A and single-trait multiple interval mapping (MIM) of three *Pgt* races TMLKC, TTKSK, and TRTTF infection type (IT). The simple sequence repeats (SSR) markers are represented with *red font* and the semi-thermal asymmetric reverse PCR (STARP) marker in light *blue font*. The QTL associated with these three *Pgt* races is designated as the *QSr.fcu-6A*.

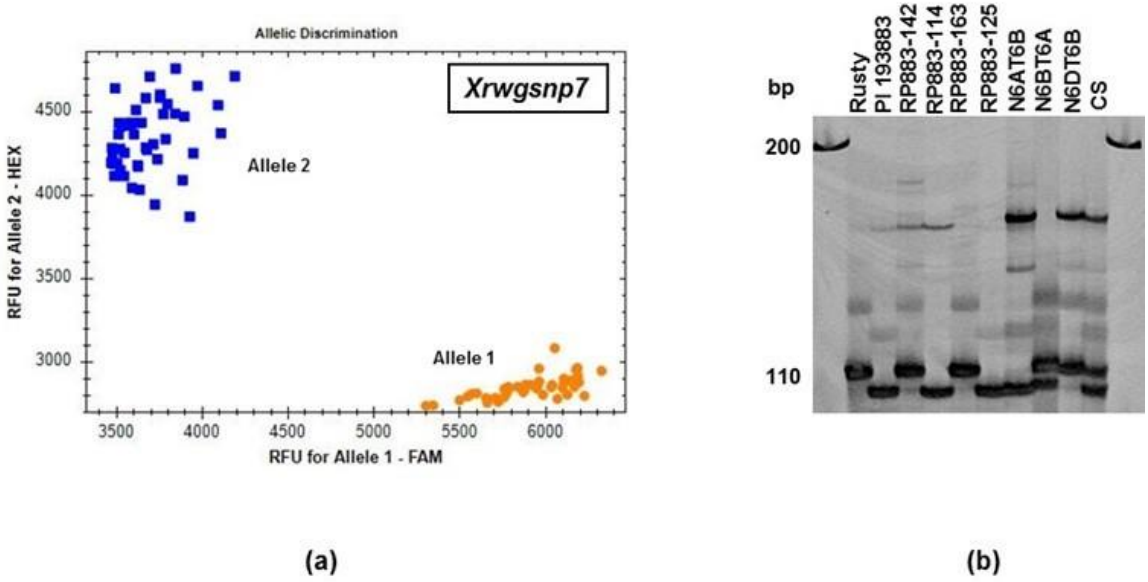

Fig 4.4. The semi-thermal asymmetric reverse PCR (STARP) marker *Xrwgsnp7* analysis using a (a) relative fluorescence unit plot with the CFX84 TouchTM Real-Time PCR detection system. Where, Allele 1 is associated with *T. turgidum* ssp. *dicoccum* accession PI 193883 and allele 2 is associated with durum wheat line Rusty. (b) *Xrwgsnp7* PCR product electrophoresis on a 6% polyacrylamide gel for parents (Rusty and PI 193883), four RILs (RP883-143, 114, 163, and 125), Chinese Spring (CS) homoeologous chromosome group 6 nullisomic-tetrasomic lines (N6AT6B, N6BT6A, and N6DT6B), and CS.

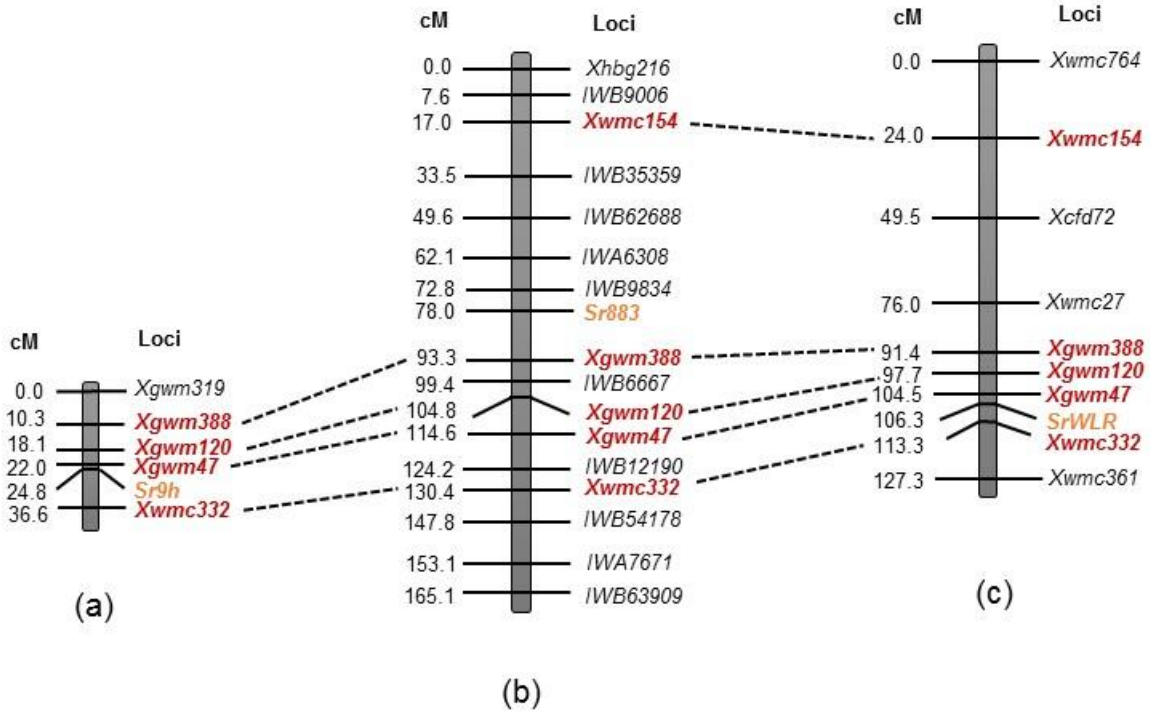

Fig 4.5. Comparison of *Sr883* region with other known *Sr* genes on the chromosome arm 2BL (a) *Sr9h* mapped in Gabo 56/Chinese Spring (Rouse et al. 2014) (b) *Sr883* mapped in Rusty/ PI 193883 (c) *SrWLR* mapped in LMPG-6/ spring wheat landrace PI 626573 (Zurn et al. 2014). The simple sequence repeats (SSR) markers are represented with *red font* and *Sr* genes in *orange font*.

Table 4.7. Grouping of RILs based on allelic states at *QSr.fcu-2B* and *QSr.fcu-6A* flanking markers regions

Genotype[a]	Class	TTKSK			TRTTF			TMLKC		
		No.[b]	IT[c]	SD[d]	No.[b]	IT[c]	SD[d]	No.[b]	IT[c]	SD[d]
QSr.fcu-2B	1	33	5.5[c]	0.7	30	5.0[c]	0.4	29	6.4[b]	1.5
QSr.fcu-6A	2	41	8.1[b]	1.0	37	8.1[b]	0.8	42	6.7[b]	1.3
QSr.fcu-2B + *QSr.fcu-6A*	3	30	5.6[c]	0.7	30	4.9[c]	0.4	30	4.3[c]	1.7
None	4	44	9.0[a]	0.2	44	9.0[a]	0.2	47	8.9[a]	0.4

[a]Genotype: alleles from PI 193883 in the 2B and 6A QTL region
[b]No. = Number of RILs
[c]Mean= Means infection type (IT) values with different letters are significantly differ from each other
[d]SD = Standard deviation

4.4. Discussion

The current study showed that PI 193883 conferred resistance against *Pgt* races TTKSK, TRTTF, and TMLKC with IT type "2 to 22-". Olivera et al. (2012) also reported PI 193883 to be resistant against *Pgt* races TTKSK and TRTTF, as well as TTTTF, TPMKC, RKQQC, QTHJC, and MCCFC. The Olivera et al. (2012) study was conducted using Cltr 7966 x PI 193883 F_1 and F_2 plants and showed recessive gene inheritance for resistance to TTKSK, TRTTF, and TTTTF. However, Rusty × PI 193883 derived F_1 plants showed variation in the phenotypic scores for *Pgt* races: susceptible to TTKSK and TRTTF, and resistant to TMLKC (Table 4.1). This indicates the presence of more than one gene or perhaps a different mode of resistance. Furthermore, data from stem rust screening of F_2 plants for TMLKC and TRTTF also suggests that there is more than one gene conferring resistance against these *Pgt* races (Table 4.1).

QTL analysis of the RP883 population showed two major QTL for resistance to stem rust disease caused by TTKSK, TRTTF, and TMLKC. *QSr.fcu-2B* on 2BL provides resistance against three races used in the current study. The *QSr.fcu-2B* region is located proximal to *Xgwm388*, whereas *Sr9* (Rouse et al. 2014) and *SrWLR* mapped distal to that marker. This suggests that *QSr.fcu-2B* is likely due to the presence of a novel *Sr* gene.

Another QTL identified in the current study, *QSr.fcu-6A*, conferred different levels of resistance against TMLKC, TTKSK, and TRTTF. Based on the chromosomal location of SSR marker *Xbarc104*, *QSr.fcu-6A* is located in the region known to be associated with *Sr13* (McIntosh 1995; Periyannan et al. 2014; Simons et al. 2011). *Sr13* is known to provide resistance against the three *Pgt* races used in the current study. However, *QSr.fcu-6A* region explained more variation for TMLKC than the other two *Pgt* races (Table 4.2). In addition, QTL position for TTKSK and TRTTF (136 cM) is slightly shifted compared to TMLKC (138 cM), and the

average IT score of RILs with only *QSr.fcu-6A* is also different for TMLKC than the other two races (Table 4.7). Another study conducted in the Dr. Steven. S. Xu lab (unpublished) on the RP883 parental lines, Rusty and PI 193883, and eight wheat lines containing *Sr13* or *Sr13* alleles with the STARP marker *Xrwgsnp7* and multiple *Pgt* races revealed that probably PI 193883 does not carry the same *Sr13* gene or allele as the other tested lines. Therefore, the possibility of two distinct but closely associated genes can not be rule out in this situation.

The four genotypic classes had a different average IT score for TTKSK and TRTTF than they did for TMLKC (Table 4.7). Examining class 1 and class 3 RILs indicates that *QSr.fcu-2B* is epistatic to *QSr.fcu-6A*. F_1 plants showed susceptible reaction type for TTKSK and TRTTF, which suggests that *Sr883* is a recessive gene. Hence, it is possible that a recessive epistasis is operating between both QTL regions for *Pgt* races TTKSK and TRTTF. In contrast, for TMLKC, RILs in class 1 and 2 display the same level of resistance, whereas there is an increase in the resistance level for the class 3 RILs (Table 4.7, Fig. 4.1). The F_1 plants showed a dominant reaction type against TMLKC, which suggest that *QSr.fcu-6A* is inherited dominantly (Table 4.7, Fig. 4.1). However as I mentioned earlier, it is not confirmed whether one or two *Sr* genes reside in the *QSr.fcu-6A* region. If I assume that only one *Sr* gene conferred the resistance against the three races, than it is presumbably a dominat allele's contradication taking place between the two QTL regions. A second possibility is that there are two loci on chromosome arm 6AL: one conditioning resistance for TTKSK and TRTTF, while the other for TMLKC. If that is the situation then certainly *QSr.fcu-2B* and *QSr.fcu-6A* loci had an additive effect for TMLKC.

In conclusion, both QTL regions look promising for future improvement of wheat cultivars due to their effectiveness against multiple *Pgt* races. The newly developed STARP and SSR markers will be fruitful for pyramiding of genes and MAS breeding. Because there is

interaction going on between the two cultivated emmer PI 193883 derived *Sr* genes, it will be important to understand the mechanisms underlying their function for the future deployment of these two *Sr* genes in durable resistance breeding programs.

4.5. References

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5. IDENTIFICATION AND MAPPING OF STEM RUST RESISTANCE GENES IN ADAPTED U.S. DURUM WHEAT GERMPLASM

5.1. Introduction

The disease stem rust, caused by *Puccinia graminis* Pers.:Pers. f. sp. *tritici* Eriks. & E. Henn. (*Pgt*), constantly threatens worldwide production of bread wheat (*Triticum aestivum* L., 2*n* = 6*x* = 42, AABBDD) and durum wheat (*T. turgidium* ssp. *durum* (Desf.) Husnot, 2*n* = 4*x* = 28, AABB) due to adaptation of *Pgt* populations to the deployed stem rust resistance (*Sr*) genes. Durum wheat is one of the major crops produced in the upper Great Plains in North America; North Dakota (ND) alone contributes 67% of the total U.S. durum wheat production (Elias and Manthey 2012). Historical data indicate that durum and bread wheat crops in the upper Great Plains region are highly vulnerable to stem rust outbreaks (Roelfs 1978). Since 1904, nine outbreaks of stem rust have been reported on wheat in this region (Paarlberg et al. 2014). During the 1930's and 1950's, the epidemics caused by *Pgt* races 56 (MCCFC) and 15B (TMLKC/TPMKC) (https://www.ars.usda.gov/SP2UserFiles/ad_hoc/36400500Cerealrusts/Pgtraceconversions.xls) led to significant yield losses of the durum and spring wheat crops in Minnesota and North Dakota (Roelfs 1978; Dubin and Brennan 2009; Paarlberg et al. 2014). Consequently, stem rust resistance has been one of the major targets for durum and spring wheat breeding programs in this region since the 1910's (Hayes et al. 1936). Certain *Sr* genes that are effective against the prevailing races have been deployed into durum and bread wheat varieties. Such efforts in breeding for stem rust resistance have led to the development of wheat varieties that carry several *Sr* genes.

The *Pgt* race TTKSK and its 12 variants (commonly known as the Ug99 race group) originated from East Africa and are currently considered a major threat to world wheat production due to their virulence on many deployed *Sr* genes (Pretorius et al. 2000; Singh et al. 2011, 2015; Fetch et al. 2016). Approximately half of the 70 known *Sr* genes are ineffective against the Ug99 race group, including the widely deployed *Sr24*, *Sr31*, *Sr36*, and *Sr38* genes (McIntosh et al. 2013, 2014; Singh et al. 2015). At present, more than 80% of worldwide wheat production is under the potential threat of the Ug99 race group (Singh et al. 2011; Lopez-Vera et al. 2014).

In addition to the Ug99 race group, several other *Pgt* races have raised concerns due to their broad virulence against frequently deployed *Sr* genes. The races TRTTF, JRCQC, and TTTTF were identified in Yemen (2006), Ethiopia (2009), and the U.S. (2000), respectively (Jin 2005; Olivera et al. 2012). These races were reported as virulent to *Sr9e* and/or *Sr13*, which are the major sources of resistance in North American and CIMMYT durum wheat varieties (Jin 2005; Periyannan et al. 2014; Singh et al. 2015). In addition, TRTTF is virulent on *Sr36*, *SrTmp*, and $Sr1RS^{Amigo}$, which are present in many U.S. winter wheat varieties (Jin and Singh 2006; Olivera et al. 2012), and TTTTF is broadly virulent to *Sr* genes in the North American stem rust differential set, including *Sr36* and *SrTmp* (Jin 2005), and North American winter wheat germplasm. Another non-Ug99 lineage *Pgt* race of concern is TKTTF, which was identified in Ethiopia and Germany in 2013 (Olivera et al. 2015, 2017). However, the TKTTF isolates from both locations are phenotypically different from each other (Olivera et al. 2017). TKTTF was responsible for a localized epidemic in Ethiopia, which led to nearly 100% crop loss of the popular variety Digalu in the southern region of the country (Olivera et al. 2015; Turner et al. 2016). Therefore, detection of highly virulent *Pgt* races in the last two decades demonstrates a

vulnerability in the adapted cultivars and the need for new sources of resistance. Currently, at least three non-Ug99 lineage races (TRTTF, JRCQC, and TTTTF) are potential threats to durum and winter bread wheat production in North America.

The resistance to TTKSK in the durum wheat varieties and germplasm adapted to the upper Great Plains of the U.S. is primarily due to the presence of *Sr13* derived from Khapli emmer wheat [*T. turgidum* ssp. *dicoccum* (Schrank ex Schubler) Thell.] (Simons et al. 2011). Most recently, Nirmala et al. (2017) reported that most of the U.S. Great Plains durum varieties also carry an *Sr* gene (temporarily designated as *Sr8155B1*) located in the *Sr8* region of chromosome arm 6AS. This gene is unique due to its resistance against the *Pgt* race TTKST and other variants, although it was not effective against the first-detected race TTKSK in the Ug99 race group. Furthermore, it has been found out recently that all the ND durum varieties and breeding lines tested were also highly resistant to TRTTF, indicating that they may carry additional uncharacterized *Sr* genes. As current varieties and adapted germplasm are usually used as parents for developing new varieties, knowledge of the *Sr* genes already present in the varieties and adapted germplasm is essential for the selection of donor *Sr* genes and breeding schemes suitable for gene pyramiding and deployment. It has been recognized that pyramiding, or combining more than one resistance (R) gene, is the most promising strategy to achieve broad-spectrum, durable resistance against multiple races of a single pathogen or pest (Singh et al. 2011).

Marker-assisted selection (MAS) is frequently used to pyramid multiple genes in wheat lines. Various molecular markers have been used for MAS breeding, but in the last few years, SNP genotyping technologies have made significant progress in agricultural research. Recently, Long et al. (2017) developed a novel SNP genotyping method known as semi-thermal

asymmetric reverse PCR (STARP). This method is advantageous for marker-assisted selection (MAS), due to high accuracy, low operation costs, and its adaptability to different genotyping platforms (Klindworth et al. 2017; Long et al. 2017). Therefore, development of STARP markers linked to the major *Sr* genes will facilitate gene pyramiding and deployment for improving stem rust resistance in wheat breeding.

Among the major durum varieties in production in North Dakota, 'Lebsock' (Elias et al. 2001) was the leading variety in the state for five years from 2004 (27.9%) to 2008 (26.7%) (USDA-NASS 2006, 2009). Although Lebsock production is reduced now due to its susceptibility to Fusarium head blight (USDA-NASS 2017), it has been used as a parent for developing new durum germplasm and populations. A doubled haploid (DH) population designated as LP749 derived from a hybrid between Lebsock and *T. turgidium* ssp. *carthlicum* (Nevski in Kom.) Á.Löve & D.Löve (2*n* = 4*x* = 28, AABB) accession PI 94749 and its SSR-based linkage map have already been developed (Chu et al. 2010). This DH population and the linkage map were previously used for the identification of novel quantitative trait loci (QTL) for tan spot resistance (Chu et al. 2010), a novel *Vrn-B1* allele (*Vrn-B1c*) for plant growth habitat (Chu et al. 2011), and a new sensitivity gene (*Snn5*) for a necrotrophic effector (SnTox5) produced by *Parastagonospora* (syn. *ana*, *Stagonospora*; *teleo*, *Phaeosphaeria*) *nodorum* (Berk.) (Friesen et al. 2012). The parents of the LP749 population, Lebsock and PI 94749, differ in their resistance against *Pgt*. I hypothesized that the LP749 population will segregate for stem rust resistance, and therefore screened the DH population with *Pgt* races TTKSK, TRTTF, and TTTTF to identify QTL associated with stem rust resistance derived from Lebsock. Additionally, allele-specific STARP markers for the *Sr* regions were developed to facilitate the pyramiding of these genes in modern varieties.

5.2. Materials and methods

5.2.1. Plant materials

Nine durum wheat varieties in addition to a population of 146 tetraploid DH lines derived from the F_1 hybrids between durum wheat variety Lebsock and *T. turgidium* ssp. *carthlicum* accession PI 94749 were analyzed for their stem rust reaction. The nine durum wheat varieties including 'Joppa', 'Carpio', 'Tioga', 'Alkabo', 'Divide', 'Grenora', Lebsock, 'Maier', and 'Ben', were developed by the North Dakota Agricultural Experiment Station in cooperation with the USDA-ARS and released from 1996 to 2014 (Elias et al. 2001, 2014; Elias and Manthey 2007a, b, c, 2016; Elias and Miller 1998, 2000). In 2015, these varieties accounted for over 40% and 76.5% of the durum acreage planted in Montana (USDA-NASS 2015a) and North Dakota (USDA-NASS 2015b), respectively. The development of the LP749 population was previously described (Chu et al. 2010).

5.2.2. Stem rust analysis

The nine durum wheat varieties and the LP749 population were evaluated for reactions to three *Pgt* races: TTKSK (isolate 04KEN156/04), TRTTF (06YEM34-1), and TTTTF (01MN84A-1-2), at the USDA-ARS Cereal Disease Laboratory, St. Paul, MN. Stem rust evaluations were performed at the seedling stage using a method described by Rouse et al. (2011). The avirulence/virulence profiles of these three races on the North America differentials are listed in Table 5.1. The evaluation of the 146 DH lines in the LP749 population and parents (Lebsock and PI 94749) was conducted in two biological replications. For each replication, five seeds were used for individual DH lines. The F_1 plants were also tested for stem rust response. Plants were scored for infection type (IT) using the Stakman et al. (1962) scale, where basic ITs were "0, ; , 1, 2, 3, and 4". The additional symbols "$^+$" and "$^-$" were used to represent large and

small pustules within an IT, respectively (Roelfs and Martens 1988). Plants with an IT score 0-2 were considered resistant, and those with a score of 3-4 were considered susceptible. For QTL analysis, the IT scores of individual races were converted using a scale of 0 to 9 based on the method described by Zhang et al. (2014). The mean of converted scores for each line in response to individual *Pgt* races from two replications was used in the QTL analysis.

Table 5.1. Avirulence and virulence of three races of *Puccinia graminis* f. sp. *tritici* (*Pgt*) to the North American differentials

Pgt race (isolate)	Avirulent	Virulent
TTKSK (04KEN156/04)	*Sr24 36 Tmp*	*Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 31 38 McN*
TRTTF (06YEM34-1)	*Sr8a 24 31*	*Sr5 6 7b 9a 9b 9d 9e* [a] *9g 10 11 17 21 30 36 38 McN Tmp*
TTTTF (01MN84A-1-2)	*Sr24 31*	*Sr5 6 7b 8a 9a 9b 9d 9e 9g 10 11 17 21 30 36 38 McN Tmp*

[a] Virulence of TRTTF to *9e* is variable due to the minor effect.

5.2.3. Marker analysis

The LP749 population linkage maps, comprised of SSRs, were used for the initial QTL analysis (Chu et al. 2010). To further saturate the current linkage maps, additional SSRs that were previously reported from chromosome arms 4AL and 6AS were analyzed. Additionally, allele-specific STARP markers were developed for these two regions. For both approaches, genomic DNA was extracted from the 146 DH lines and the parents using the method described by Faris et al. (2000). For SSR analysis, 16 molecular markers selected from different maps (Somers et al. 2004; Sourdille et al. 2004; Torada et al. 2006; Xue et al. 2008; Zhang et al. 2008; Sorrells et al. 2011) were further mapped on the linkage groups in this study. Of these newly selected markers, 11 and five were mapped on chromosomes 4A and 6A, respectively. Of the 11

molecular markers on 4A, six were SSRs *Xgwm397*, *Xgwm637*, *Xbarc135*, *Xcfd31*, *Xwmc776*, and *Xhbg452* and the remaining five were eSTS markers *Xmag3886*, *Xmag3733*, *Xmag3092*, *Xmag1574*, and *Xmag1604*. The five newly mapped markers on chromosome 6A were SSRs *Xgpw2295*, *Xhbe302*, *Xhbg239*, *Xhbg347*, and *Xhbe297*. For marker assays, polymerase chain reactions (PCR) were performed by following the procedure in Röder et al. (1998). The amplified PCR products were electrophoresed on 6% non-denaturing polyacrylamide gels. Gels were stained with Gelred™ nucleic acid stain (Biotium Corporate, Hayward, CA), and a Typhoon 9410 variable mode imager (GE healthcare Biosciences, Waukesha, WI) was used to capture images.

To develop STARP markers for the 4AL region, SNPs were selected from a 9K-based *Sr7a*-associated 4AL linkage map reported by Turner et al. (2016). In this map, two SNPs, *IWA1066* and *IWA106*, were located near the *Sr7a* region. These SNPs were used to select additional SNPs from the 90K consensus map (Wang et al. 2014). To develop STARP markers for the 6AS QTL region, SNPs were selected from a 90K-based chromosome 6A linkage map of a tetraploid RIL population (RP883) developed from a cross between durum wheat and cultivated emmer wheat (Chapter 3). The selection was made based on a common SSR *Xhbe302* between the chromosome 6A linkage maps of LP749 and RP883 populations. Flanking region sequences for the selected SNPs (Wang et al. 2014) were used as BLASTn queries (Altschul et al. 1997) to search the wheat genome scaffolds (https://urgi.versailles.inra.fr/blast_iwgsc/blast.php) to identify low copy sequences. After selection, the flanking genomic sequences of the selected SNPs were used to design the asymmetrically modified allele-specific (AMAS) and reverse primers according to the method described by Long et al. (2017). The two universal priming element-adjustable (PEA) primers

were used as reported in Long et al. (2017). The program Primer-BLAST was used for the calculation of primer's T_m values (Ye et al. 2012). PCR was conducted as described in Long et al. (2017). Thirty-three cycles were used for gel-based detection of PCR products, whereas 46 cycles were used for gel-free detection. For the gel-based system, STARP markers were evaluated as described earlier for SSR markers. However, for gel-free marker analysis, the CFX384 Touch™ Real-Time PCR detection system (Bio-Rad Laboratories, Inc., Foster City, CA, USA) was used.

To evaluate the potential of the newly developed STARP markers for MAS, a validation analysis was done using a diverse set of 50 durum and bread wheat varieties and lines. This validation panel was comprised of 18 durum and 32 common wheat varieties and lines from Australia, Brazil, Canada, China, Italy, and the U.S. One of the durum lines, 8155-B1, is monogenic for stem rust resistance derived from a durum accession C.I. 8155 (Williams and Gough 1968; Nirmala et al. 2017). In addition, the common wheat line ISr8a-Ra, which is monogenic for *Sr8a* (Jin et al. 2007), the common wheat variety 'Chinese Spring' (CS), and three CS nullisomic-tetrasomic lines involving homoeologous group 6 chromosomes (N6AT6B, N6BT6A, and N6DT6B) were used to validate the new STARP markers on chromosome arm 6AS.

5.2.4. Linkage and QTL analysis

The MapDisto 1.7.5 (Lorieux 2012) software package was used for linkage analysis. To find the grouping of new markers with the previously developed genetic maps, a logarithm of odds (LOD) 3.0 and an Rmax value = 3.0 were used. After confirming the grouping, the best order of the linkage groups was obtained using the 'order sequence', 'check inversions', 'ripple order', and 'drop locus' commands in consecutive order. The Kosambi mapping function

(Kosambi 1943) was used to calculate genetic distances. Linkage maps of the LP749 population and stem rust screening data were used to identify genomic regions associated with resistance to *Pgt* races TTKSK, TRTTF, and TTTTF. QTL analysis was conducted using QGENE (4.3.10) (Joehanes and Nelson 2008). A significant LOD threshold of 3.0 was determined by performing a permutation test consisting of 1000 permutations, and the coefficient of determination (R^2) × 100 was calculated and used to determine the amount of phenotypic variation explained by the QTL.

5.3. Results

The nine durum varieties, the LP749 population, *T. turgidium* ssp. *carthlicum* PI 94749, and the F_1 hybrid (Lebsock/PI 94749) were screened with *Pgt* races TTKSK, TRTTF, and TTTTF at the seedling stage. For TRTTF, the nine durum varieties showed nearly immune to moderately resistant (IT ; to 2^-) phenotypes (Table 5.2). For TTKSK and TTTTF, eight varieties were moderately resistant and nearly immune, respectively, but one variety (Divide) was susceptible to both races (Table 5.2). Contrary to the durum varieties, PI 94749 was susceptible to all three races. The F_1 plants (Lebsock/PI 94749) had a similar level of resistance to the three races as Lebsock, suggesting that the *Sr* genes in Lebsock are of dominant nature (Table 5.2). Among the 146 DH lines, 145, 143, and 139 lines were evaluated for reactions to TTKSK, TRTTF, and TTTTF, respectively (Table 5.3). For TTKSK, the LP749 population segregated into 76 resistant to 69 susceptible; and, for TTKSK and TRTTF, the population segregated into 124 resistant to 19 susceptible and 98 resistant to 41 susceptible, respectively (Table 5.3). A Chi-squared goodness-of-fit test showed that segregation for TTKSK reaction fit a 1:1 ratio (χ^2 = 0.34, P = 0.56), indicating the presence of a single gene for TTKSK resistance (Table 5.3). On

the other hand, segregation of reaction to TTTTF fit a two-gene ratio of 3:1 (χ^2 = 1.5, *P* = 0.22), and segregation of reaction to TRTTF fit a three-gene ratio of 7:1 (χ^2 = 0.08, *P* = 0.78).

Table 5.2. Infection types produced by nine durum wheat varieties, *T. turgidum* ssp. *carthlicum* PI 94749, and Lebsock × PI 94749-derived F_1 plants to three races of *P. graminis* f. sp. *tritici*

Variety or accession	Species or Pedigree	Year released	TTKSK	TRTTF	TTTTF	Putative *Sr* gene or allele[a]
Joppa	Maier/D97643	2014	2-	;/2-	;	*Sr7a*, *Sr13*, *Sr8155B1*
Carpio	D95580/D95595	2012	2-	2-	;	*Sr7a*, *Sr13*
Tioga	Plaza/Maier	2010	2-	2-	;	*Sr7a*, *Sr13*
Alkabo	D901247/D89263	2005	2	0	;	*Sr7a*, *Sr13*, *Sr8155B1*
Divide	Ben/D901282//Belzer	2005	4	0;	3-	*Sr8155B1*
Grenora	D901260/D901419	2005	2	0	;	*Sr7a*, *Sr13*, *Sr8155B1*
Lebsock	Munich/D8469	1999	2-	0;	;	*Sr7a*, *Sr9e*, *Sr13*, *Sr8155B1*
Maier	D8193/D8335	1998	2-	0;	;	*Sr7a*, *Sr13*, *Sr8155B1*
Ben	D8024/Monroe	1996	2-	0;	;	*Sr7a*, *Sr13*, *Sr8155B1*
PI 94749	*T. turgidum* ssp. *carthlicum*		3+3	3+	3+3	
LP749 (F_1)	Lebsock/PI 94749		23;	;1	;12-	

[a] Putative *Sr* genes or alleles in durum varieties were determined based on mapping, marker genotyping, and race-specification analysis conducted in this study. Joppa is heterogeneous for *Sr8155B1*. The allele *Sr9e* in Lebsock was determined based on mapping analysis and its presence in all other varieties could not be determined due to its minor effect. Presence of *Sr8155B1* in Divide, Grenora, and Alkabo was recently reported by Nirmala et al. (2017)

Table 5.3. Chi-squared analysis of segregation of resistance to three races of the stem rust pathogen in a doubled haploid (DH) population derived from hybrid between durum 'Lebsock' and *T. turgidum* ssp. *carthlicum* PI 94749

	No. of DH lines[a]			χ^2			Probability		
Race	Total	R	S	(1:1)	(3:1)	(7:1)	(1:1)	(3:1)	(7:1)
TTKSK	145	76	69	0.34*			0.56		
TRTTF	143	124	19			0.08*			0.78
TTTTF	139	98	41		1.50*			0.22	

[a] No. of DH line: Total = total number of DH lines evaluated, R = resistant, S = susceptible

Four QTL were associated with the Lebsock-derived resistance against the *Pgt* races, and they were located on chromosome arms 2BL, 4AL, 6AS, and 6AL (Fig. 5.1). The 2BL QTL associated with TRTTF resistance had an LOD of 5.27 and was designated as *QSr.rwg-2B*. This QTL was flanked by SSR markers *Xgwm16* and *Xwmc175* and explained 4% of the phenotypic variation for resistance to TRTTF (Table 5.4). Based on the chromosomal location of SSR marker *Xwmc175*, *QSr.rwg-2B* resistance was most probably conferred by an allele of *Sr9* (Rouse et al. 2014). Several alleles of *Sr9*, including *Sr9a*, *Sr9b*, *Sr9d*, and *Sr9g*, are ineffective against TRTTF (Table 5.1), whereas *Sr9e* has a minor effect for resistance to TRTTF (M.N. Rouse, Y. Jin, unpublished). Considering the minor effect of *QSr.rwg-2B* on resistance to TRTTF and prevalence of *Sr9e* in North American durum varieties, I speculate that the gene underlying this QTL is possibly *Sr9e*.

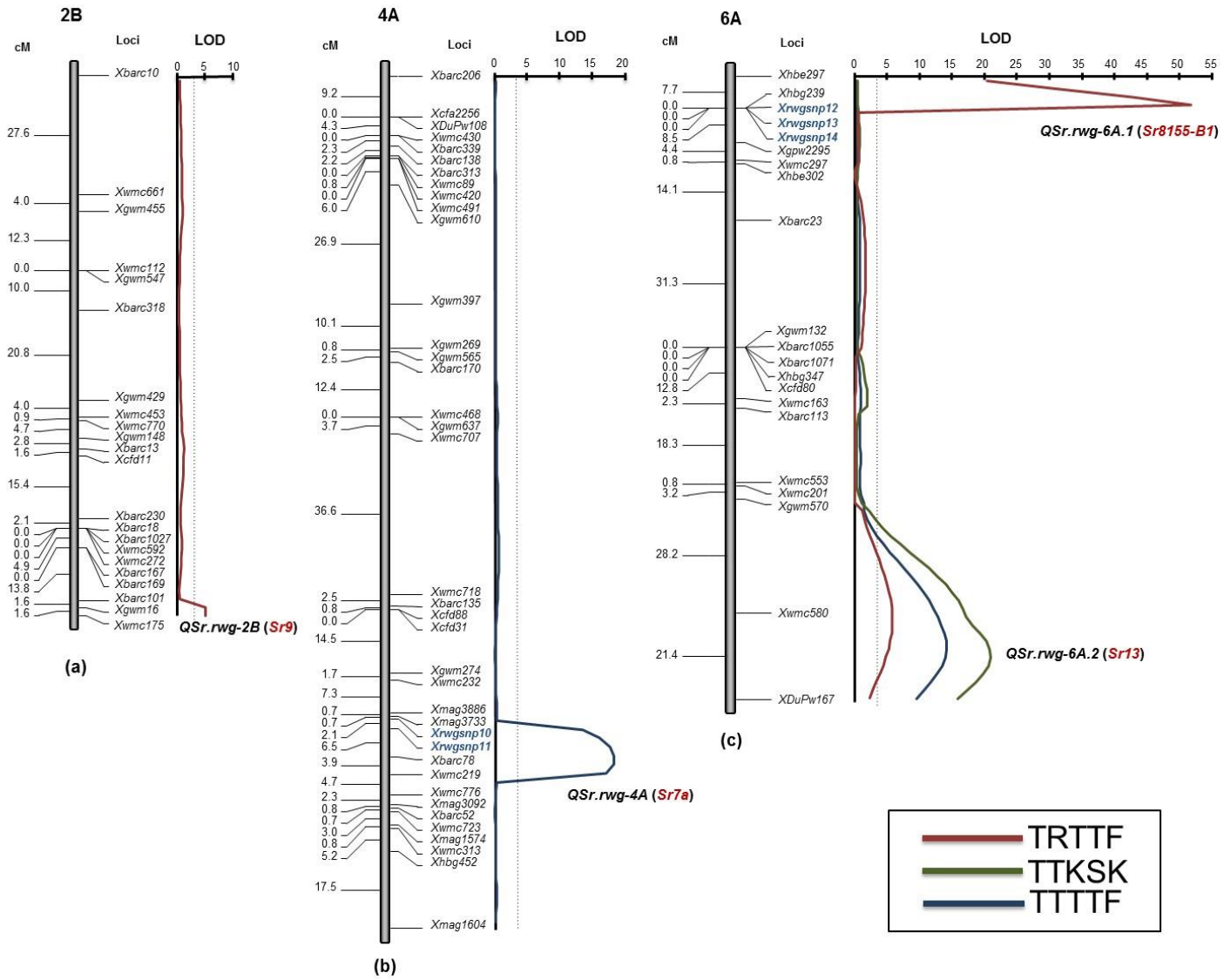

Fig. 5.1. Genetic map and composite interval mapping of the chromosome 2B, 4A, and 6A representing *QSr.rwg-2B*, *QSr.rwg-4A*, *QSr.rwg-6A.1*, and *QSr.rwg-6A.2*. The dashed lines represent the threshold LOD = 3.0. The putative genes associated with the QTL regions are shown in red font.

The QTL on chromosome arm 4AL, designated *QSr.rwg-4A*, had an LOD of 18.12 and explained 37% of the variation associated with resistance to TTTTF (Table 5.4). This QTL spanned a 13-cM interval between markers *Xmag3886* and *Xwmc219*. This region is known to be associated with the deletion bin 4AL-4 (Sourdille et al. 2004). *QSr.rwg-4A* most likely corresponded to *Sr7a* based on the chromosomal location of the *Sr7* locus, the unique avirulence of *Sr7a* to TTTTF, and the parentage of Lebsock (Elias et al. 2001). Among the other eight durum varieties evaluated this study, only Divide was susceptible to TTTTF (IT 3^{-}), indicating that Divide doesn't carry *Sr7a* (Table 5.2). However, other seven varieties all had the same IT (;) as Lebsock, suggesting that they most likely all have this gene (Table 5.2).

Table 5.4. QTLs associated with seedling resistance to stem rust caused by *Puccinia graminis* races TRTTF, TTKSK, and TTTTF detected by composite interval mapping. The chromosome arm locations, putative *Sr* gene, associated markers, LOD, R^2, and additive effects are given

QTL	Chr.[a]	Marker interval	Putative *Sr* gene or allele	TRTTF			TTKSK			TTTTF		
				LOD	R^2	Add.[b]	LOD	R^2	Add.	LOD	R^2	Add.
QSr.rwg-2B	2BL	*Xgwm16- Xwmc175*	*Sr9e*	5.27	0.04	-0.51	-[c]	-	-	-	-	-
QSr.rwg-4A	4AL	*Xmag3886 - Xwmc219*	*Sr7a*	-	-	-	-	-	-	18.12	0.37	-1.91
QSr.rwg-6A.1	6AS	*Xhbe297- Xgpw2295*	*Sr8155B1*	51.63	0.81	-3.17	-	-	-	-	-	-
QSr.rwg-6A.2	6AL	*Xwmc580- XDuPw167*	*Sr13*	5.84	0.08	-0.54	20.70	0.46	-2.49	13.87	0.28	-1.79

[a]Chr.: Chromosome arm, [b]Add.: Additive effects of the QTL, a negative value indicates resistance derived from Lebsock
[c]A symbol "-" indicates no significant association with resistance

Among the two QTL present on chromosome 6A, the QTL located on chromosome arm 6AS was specific only to *Pgt* race TRTTF (Fig. 5.1). This QTL, designated as *QSr.rwg-6A.1*, had a LOD value of 51.63 and explained 81% of the phenotypic variation associated with resistance to TRTTF (Table 5.4). *QSr.rwg-6A.1* was flanked by the SSR markers *Xhbe297* and *Xgpw2295* (Fig. 5.1) in the distal deletion bin 6AL-5. The map location and uniquely high level of resistance to TRTTF suggested that *QSr.rwg-6A.1* corresponds to the *Sr8155B1* gene recently reported by Nirmala et al. (2017). The second QTL located on 6AL was designated as *QSr.rwg-*

6A.2 and provided resistance against all three *Pgt* races used in the current study with LOD values ranging from 5.84 to 20.70 (Table 5.4, Fig. 5.1). *QSr.rwg-6A.2* was flanked by markers *Xwmc580* and *XDuPw167* (Fig. 5.1) and explained 46%, 28%, and 8% of the variation for resistance associated with races TTKSK, TTTTF, and TRTTF, respectively (Table 5.4). Based on the molecular markers mapped in the *QSr.rwg-6A.2*-associated genomic region, this QTL is located in the distal deletion bin 6AL-8 (Fig. 5.1) and likely corresponds to the *Sr13* gene (Simons et al. 2011). As similar to the reaction to TTTTF, Divide is the only variety that was susceptible to TTKSK, other seven varieties all had the same or similar ITs (2 or 2^{-}) as Lebsock, suggesting that these TTKSK-resistant varieties most likely all have *Sr13* (Table 5.2).

Five STARP markers were mapped in the current study, out of which two markers, *Xrwgsnp10* and *Xrwgsnp11*, were mapped near the *Sr7a* region of chromosome arm 4AL (Fig. 5.1, 5.2). Three STARP markers, *Xrwgsnp12*, *Xrwgsnp13*, and *Xrwgsnp14*, were mapped in the *Sr8155B1* region on chromosome arm 6AS (Fig. 5.1, 5.2, Table 5.5). All of the markers worked effectively with the gel based system, however only *Xrwgsnp11*, *Xrwgsnp13*, and *Xrwgsnp14* worked effectively with the gel-free system (Fig. 5.2). Of the five STARP markers linked with *QSr.rwg-4A* and *QSr.rwg-6A.1*, three (*Xrwgsnp11, Xrwgsnp13*, and *Xrwgsnp14*) were co-dominantly inherited, whereas *Xrwgsnp10* and *Xrwgsnp12* were dominant in nature, with *Xrwgsnp10* being in the repulsion phase and *Xrwgsnp12* in the coupling phase (Table 5.5). The Lebsock and PI 94979 alleles were differentiated in durum and common wheat by three (*Xrwgsnp11*, *Xrwgsnp12*, and *Xrwgsnp13*) of the five STARP markers. *Xrwgsnp10* and *Xrwgsnp14* were polymorphic only in durum varieties. Additionally, *Xrwgsnp14* was able to differentiate between the different durum varieties for the *Sr8155B1* alleles but it could not differentiate *Sr8a* and *Sr8155B1* alleles (Fig. 5.3).

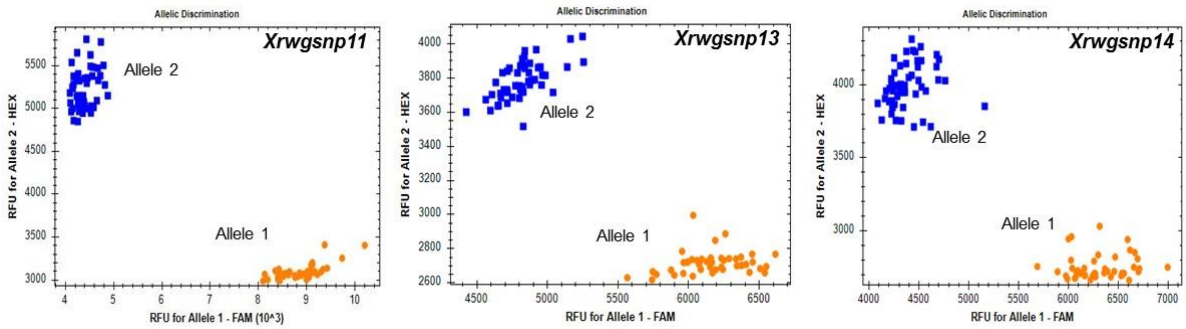

Fig. 5.2. Plots showing the clustering of the LP749 population for three STARP markers *Xrwgsnp11*, *Xrwgsnp13*, and *Xrwgsnp14* analyzed with the CFX84 TouchTM Real-Time PCR detection system. Allele 1 is associated with Lebsock, allele 2 is associated with PI 94749.

Table 5.5. The semi-thermal asymmetric reverse PCR (STARP) markers and their SNP source, sequence, product size and inheritance

Marker	Chr.[a]	Source SNP		Primers			
		ID	Name	Type	Sequence (5'-3')[b]	Product ~Size (bp)	Inheritance/phase
Xrwgsnp10	4AL	*IWB71467*	Tdurum_contig43961_607	*Xrwgsnp10*-F1 *Xrwgsnp10*-F2 *Xrwgsnp10*-R	[tail-1]TCAGGTCAGCAAACCCGT [tail-2]TCAGGTCAGCAAACTTGC CGCCGACCGTGCCTTC	36, 50	Dominant/ repulsion
Xrwgsnp11	4AL	*IWB73323*	Tdurum_contig75819_1471	*Xrwgsnp11*-F1 *Xrwgsnp11*-F2 *Xrwgsnp11*-R	[tail-2]GATGCCTCTGAAGATATATCA [tail-1]GATGCCTCTGAAGATATGCCG CAGTTTCAGATGAAAAGGCCAG	43, 53	Co-dominant
Xrwgsnp12	6AS	*IWB11274*	BS00082812_51	*Xrwgsnp12*-F1 *Xrwgsnp12*-F2 *Xrwgsnp12*-R	[tail-1]GTTCTCAGCATACACTTCTG [tail-2]GTTCTCAGCATACACTTTCA TTCGAGGTGCCGATGGTGC	100, 125	Dominant/ coupling
Xrwgsnp13	6AS	*IWB53755*	RAC875_c13610_822	*Xrwgsnp13*-F1 *Xrwgsnp13*-F2 *Xrwgsnp13*-R	[tail-2]AATCATCAGATGCTGTTTGC [tail-1]AATCATCAGATGCTGTCCGT TTGTTACGTGTATTAGAGGGGC	105, 110	Co-dominant
Xrwgsnp14	6AS	*IWB47842*	Kukri_c80373_786	*Xrwgsnp14*-F1 *Xrwgsnp14*-F2 *Xrwgsnp14*-R	[tail-1]GCCTTCTTTTCCTTGGAAACTT [tail-2]GCCTTCTTTTCCTTGGAACTTC GCTCTCAGGAACAAGTTAATTGG	105	Co-dominant

[a]Chr.: Chromosome arm, [b]Tail-1 and 2 universal sequences are 5'-GCAACAGGAACCAGCTATGAC-3'and 5'-GACGCAAGTGAGCAGTATGAC-3', respectively

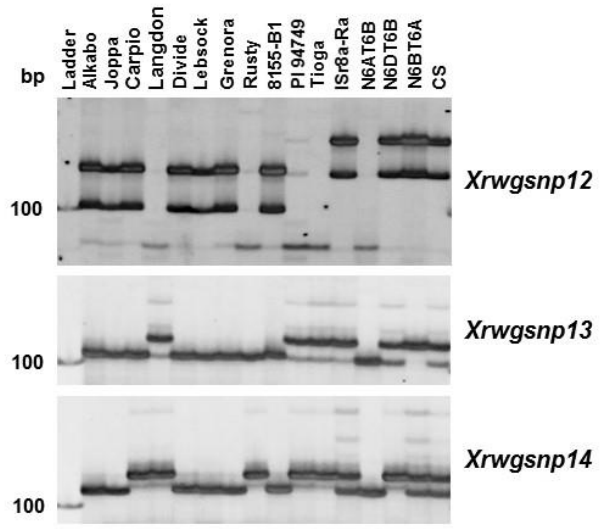

Fig. 5.3. The *QSr.rwg-6A.1* region-associated STARP markers *Xrwgsnp12*, *Xrwgsnp13*, and *Xrwgsnp14* on the different durum varieties Alkabo, Joppa, Carpio, Langdon, Divide, Lebsock, Grenora, Rusty, and Tioga, and monogenic line 8155-B1, *T. turgidum* ssp. *carthlicum* accession PI 94749, Isogenic line ISr8a-Ra, nullisomic-tetrasomic lines involving homoeologous group 6 chromosomes (N6AT6B, N6BT6A, and N6DT6B), and Chinese Spring (CS).

5.4. Discussion

North Dakota is the major producer of durum wheat in the United States. Although this region is particularly vulnerable to stem rust, most of the durum wheat varieties grown in this region are highly resistant (Elias et al. 2001; Olivera et al. 2012). Among the nine durum varieties evaluated in this study, eight are highly resistant to the U.S. race TTTTF and moderately resistant to the African race TTKSK. All nine varieties are either highly or moderately resistant to the Yemen race TRTTF. These results strongly indicated the presence of multiple *Sr* genes in ND durum varieties. It is well known that emmer wheat (*T. turgidum* ssp. *dicoccum*) lines Khapli and Vernal and the Ethiopian durum landrace ST464 (PI 191365) were used to introduce resistance into modern durum varieties (Klindworth et al. 2007; Simons et al. 2011). Several previous studies indicated that Khapli carries *Sr7a*, *Sr13*, and *Sr14*, Vernal has *Sr9e* and *Sr13*, and ST464 has *Sr9e* and *Sr13* (Knott 1962, 1996; Williams and Gough 1965;

Klindworth et al. 2007; Simons et al. 2011). In addition, both Khapli and ST464 carry additional undetermined *Sr* genes (Klindworth et al. 2007; Simons et al. 2011). However, except for *Sr13*, other *Sr* genes present in modern ND durum varieties have not been previously identified.

In the current study, four QTL associated with resistance to *Pgt* were identified in the ND durum variety Lebsock. Among these QTL, *QSr.rwg-2B*, associated with a minor effect for resistance to TRTTF, was located in the region associated with *Sr9h* and *Sr28* (Rouse et al. 2014). These genes confer resistance to TTKSK, but in the current study, *QSr.rwg-2B* was not associated with the TTKSK resistance. Based on the avirulence/virulence profile (Table 5.1), race TRTTF is virulent on *Sr9a*, *Sr9b*, *Sr9d*, and *Sr9g*. Therefore, *Sr28* and any of these *Sr9* alleles can be ruled out as the genes underlying *QSr.rwg-2B*. Although *Sr9e* is classified as susceptible to TRTTF, it does have some minor effect that is noticeable (M.N. Rouse, Y. Jin unpublished). *Sr9e* is derived from the emmer wheat line Vernal, which is in the parentage of Lebsock and many other currently grown North American durum varieties (Simons et al. 2013; Elias et al. 2001). Because *Sr9e* has been extensively deployed in North American durum varieties (Jin 2005; Olivera et al. 2012), it is most likely the gene underlying *QSr.rwg-2B*. However, I cannot rule out the possibility that this QTL was controlled by an uncharacterized new *Sr9* allele, or a new gene linked to the *Sr9* locus. If *Sr9e* is the gene underlying *QSr.rwg-2B* in Lebsock, its presence in all other varieties (Table 5.2) investigated in this study could not be determined due to its minor effect on resistance to TRTTF (Fig. 5.1 and Table 5.4).

The race TTTTF-specific QTL *QSr.rwg-4A* was identified in the *Sr7* region of chromosome arm 4AL. The *Sr7* locus is known to have two alleles, *Sr7a* and *Sr7b* (McIntosh et al. 1995). The three *Pgt* races used in this study are virulent against *Sr7b* (Table 5.1). The *Sr7a* reaction response to TRTTF is not yet characterized; however, it was reported to be ineffective

against TTKSK (Jin et al. 2007) and confers resistance against TTTTF in hard red winter wheat 'Jagger' (Turner et al. 2016). Additionally, the presence of *Sr7a* in the old ND durum wheat variety 'Langdon' suggests it might be present in modern durum varieties. Therefore, *QSr.rwg-4A* most likely corresponds to *Sr7a* in Lebsock. Basnet et al. (2015) recently mapped *SrND643* to the *Sr7* region of hexaploid wheat, but *QSr.rwg-4A* and *SrND643* differ in their reaction to TTKSK, so these two genes are clearly not the same.

The TRTTF-specific QTL *QSr.rwg-6A.1* was identified at the distal end of chromosome arm 6AS. Among the known *Sr* genes in wheat, only *Sr8* is located in this region (Knott and Anderson 1956; Sears et al. 1957; McIntosh 1972; Singh and McIntosh 1986; Bhavani et al. 2008; Chhetri et al. 2016; Dunckel et al. 2015). Two alleles (*Sr8a* and *Sr8b*) were previously identified at the *Sr8* locus (McIntosh 1995). However, Nirmala et al. (2017) recently reported the mapping of a putative new allele designated as *Sr8155B1*. The two *Sr8* alleles and *Sr8155B1* are known to be ineffective against TTKSK, but alleles *Sr8a* and *Sr8155B1* are effective against TRTTF (Jin et al. 2007; Nirmala et al. 2017) (Table 5.1). *Sr8a* and *Sr8b* phenotypically produce infection types of 2 and X (an IT type with random distribution of uredia of different sizes, see Roelfs and Martens 1988), respectively (McIntosh 1995; Jin et al. 2007; Bhavani et al. 2008). These ITs were not observed in Lebsock, LP749, or the other durum varieties tested with the three *Pgt* races, which suggests the absence of *Sr8a* and *Sr8b* alleles in the current study. *Sr8155B1* was derived from the durum wheat line 8155-B1 and carries resistance against the Ug99 variant TTKST. However, due to its ineffectiveness against race TTKSK, this gene is unique compared to other known *Sr* genes. Nirmala et al. (2017) did not report whether *Sr8155B1* was effective against TTTTF, but did report that it was ineffective against TTKSK and effective against TRTTF, which is the same as QTL *QSr.rwg-6A.1*. Furthermore, Nirmala et al.

(2017) validated the *Sr8155B1* allele in 11 durum varieties, indicating its presence in Grenora, Divide, and Alkabo, and absence in Rusty and Tioga. Interestingly, in the present study, the validation analysis of two STARP markers (*Xrwgsnp12* and *Xrwgsnp14*) associated with *QSr.rwg-6A.1* produced similar results (Table 5.6, Fig. 5.2). Even though, *Xrwgsnp13* did not differentiate the *Sr8155B1* allele in Rusty, it was effective for differentiating other durum wheat varieties (Fig. 5.3).

The marker *Xrwgsnp14* linked to *QSr.rwg-6A.1* was developed based on 90K SNP marker *IWB47842* (Table 5.5), which co-segregated with *Sr8155B1* in the Rusty × 8155-B1 F_2 population (Nirmala et al. 2017). Therefore mapping of the same SNP in both populations suggests that *IWB47842* is predicting the *Sr8155B1* allele in both studies. Additionally, in the validation analysis, Lebsock had the same sized amplicons as 8155-B1 for all three STARP markers mapped in the *QSr.rwg-6A.1* region. Hence, based on these analyses, I conclude that *QSr.rwg-6A.1* corresponds to *Sr8155B1* derived from Lebsock, and *Xrwgsnp14* detects the presence and absence of this allele in the different durum varieties (Table 5.6, Fig. 5.3). In this study, I tested eight additional durum varieties using rust tests and new STARP markers. Nirmala et al. (2017) had previously tested four of these and found *Sr8155B1* to be present in Alkabo, Divide, and Grenora, but absent in Tioga; our results agreed with their conclusion. For the four additional varieties, the rust tests and STARP markers indicated *Sr8155B1* was present in Maier and Ben, absent in Carpio, and heterogeneous in Joppa (Table 5.2, Fig. 5.3). Because Tioga and Carpio do not carry *Sr8155B1,* they exhibited only moderate resistance against TRTTF (Table 5.2).

Table 5.6. Validation of the newly developed markers using 50 durum and common wheat varieties and lines

Variety/Line	Type	Habit	Origin[a]	Amplicon (bp)[b] from markers				
				Xrwgsnp10	*Xrwgsnp11*	*Xrwgsnp12*	*Xrwgsnp13*	*Xrwgsnp14*
PI 94749	Persian wheat	Spring	Georgia	36, 50	53	-[c]	110	110
Lebsock	Durum wheat	Spring	ND, USA	-	43	100, 125	105	105
Strongfield	Durum wheat	Spring	Canada	36, 50	53	-	105	110
Transcend	Durum wheat	Spring	Canada	36, 50	53	-	105	110
Cappelli	Durum wheat	Spring	Italy	-	43	100, 125	105	110
Svevo	Durum wheat	Spring	Italy	-	43	100, 125	105	105
15FAR344-6(255)	Durum wheat	Spring	ND, USA	-	43	100, 123, 140	110	110
D09557	Durum wheat	Spring	ND, USA	-	43	-	110	110
D09690	Durum wheat	Spring	ND, USA	36, 50	53	100, 125	105	105
D101073	Durum wheat	Spring	ND, USA	36, 50	53	100, 125	105	105
Alkabo	Durum wheat	Spring	ND, USA	-	43	100, 125	105	105
Carpio	Durum wheat	Spring	ND, USA	-	43	100, 125	105	110
Divide	Durum wheat	Spring	ND, USA	36, 50	53	100, 125	105	105
Grenora	Durum wheat	Spring	ND, USA	-	43	100, 125	105	105
Joppa	Durum wheat	Spring	ND, USA	-	43	100, 125	105	105
Langdon	Durum wheat	Spring	ND, USA	36, 50	53	-	110	110
Rusty	Durum wheat	Spring	ND, USA	36, 50	53	-	105	110
8155-B1	Durum wheat	Spring	ND, USA	-	43	100, 125	105	105
Tioga	Durum wheat	Spring	ND, USA	36, 50	53	-	110	110
Line E	Common wheat	Spring	Australia	36, 50	43	100, 123, 140	110	105, 110
BR34	Common wheat	Spring	Brazil	36, 50	43	100, 125	110	105, 110
LMPG-6	Common wheat	Spring	Canada	36, 50	43	100, 125	110	105, 110
Chinese Spring	Common wheat	Spring	China	36, 50	43	100, 123, 140	110	105, 110
Jimai 22	Common wheat	Winter	China	36, 50	43	100, 125	105	105, 115
Jinqiang 5	Common wheat	Spring	China	36, 50	53	-	110	105, 111
Sumai 3	Common wheat	Spring	China	36, 50	43	100, 123, 140	110	105, 110
Yangmai 16	Common wheat	Spring	China	36, 50	53	100, 125	110	105, 110
Zhengmai 9023	Common wheat	Facultative	China	36, 50	53	-	110	105, 110
Zhoumai 27	Common wheat	Winter	China	36, 50	53	100, 125	110	105, 110
Alsen	Common wheat	Spring	ND, USA	36, 50	43	100, 125	110	105, 110
Barlow	Common wheat	Spring	ND, USA	36, 50	43	100, 125	110	105, 110
Elgin-ND	Common wheat	Spring	ND, USA	36, 50	43	100, 125	110	105, 110
Faller	Common wheat	Spring	ND, USA	36, 50	53	100, 125	110	105, 110
Glenn	Common wheat	Spring	ND, USA	36, 50	43	100, 123, 140	110	105, 111
Grandin	Common wheat	Spring	ND, USA	36, 50	53	100, 123, 140	105	105, 110
ND830	Common wheat	Spring	ND, USA	36, 50	43	100, 125	110	105, 110
ND833	Common wheat	Spring	ND, USA	36, 50	43	100, 123, 140	110	105, 111
NDHRS16-12-19	Common wheat	Spring	ND, USA	36, 50	43	100, 125	110	105, 111
Reeder	Common wheat	Spring	ND, USA	36, 50	43	100, 125	110	105, 110
Steele-ND	Common wheat	Spring	ND, USA	36, 50	43	100, 125	110	105, 111
VitPro-ND	Common wheat	Spring	ND, USA	36, 50	43	100, 125	110	105, 110
IL06-14262	Common wheat	Winter	IL, USA	36, 50	53	100, 123, 140	110	105, 111
Newton	Common wheat	Winter	KS, USA	36, 50	53	100, 123, 140	105	105, 110
Ada	Common wheat	Spring	MN, USA	36, 50	43	100, 125	110	105, 110
Bolles	Common wheat	Spring	MN, USA	36, 50	43	100, 123, 140	110	105, 110
Linkert	Common wheat	Spring	MN, USA	36, 50	43	100, 125	110	105, 110
Tom	Common wheat	Spring	MN, USA	36, 50	53	100, 125	110	105, 110
Brick	Common wheat	Spring	SD, USA	36, 50	53	-	110	105, 111
Granger	Common wheat	Spring	SD, USA	36, 50	53	-	105	105, 111
Parshall	Common wheat	Spring	SD, USA	36, 50	43	100, 125	110	105, 110
Russ	Common wheat	Spring	SD, USA	36, 50	43	100, 123, 140	110	105, 111

[a]Origin: CO, Colorado; KS, Kansas; ND, North Dakota; SD, South Dakota; MN, Minnesota; IL, Illinois; ID, Idaho

[b]Amplicon size of markers for different wheat varieties and lines in base pairs (bp)

The second chromosome 6A QTL, *QSr.rwg-6A.2*, was located in the same region known to associate with the previously mapped (Simons et al. 2011) *Sr* gene *Sr13* on chromosome arm 6AL. In the current study, *QSr.rwg-6A.2* spanned a 21.4 cM interval (Fig. 5.1) between markers *Xwmc580* and *Xdupw167*. These two markers were also reported to span the 20.4-cM region containing *Sr13* in the genetic map developed from the durum UC1113 × 'Kofa' population (Simons et al. 2011), indicating that *Sr13* is the gene underlying *QSr.rwg-6A.2* in Lebsock. *Sr13* typically has ITs ranging from 1 to 2 for most of the *Pgt* races at the seedling stage (McIntosh 1995; Periyannan et al. 2014; Simons et al. 2011). *QSr.rwg-6A.2* showed a minor (8% of the phenotypic variation) but a significant (LOD ~6) effect on resistance to TRTTF (Fig.5.1). The low phenotypic variation associated with *QSr.rwg-6A.2* (*Sr13*) was probably due to the presence of *QSr.rwg-6A.1*, whose gene conditioned IT 0; and thus masked the TRTTF resistance effects of *Sr13* and other *Sr* genes in Lebsock. I found that *QSr.rwg-6A.2* was effective against race TRTTF in contrast to a report that *Sr13* was ineffective against TRTTF (Olivera et al. 2012). However, *Sr13* was clearly effective against race TRTTF when tested at the appropriate temperatures (Zhang et al. 2017).

In conclusion, I identified *Sr7a*, *Sr13*, *Sr8155B1*, and likely *Sr9e* in the durum variety Lebsock. This study showed that the existing *Sr* genes in most of North Dakota durum varieties provide adequate protection from the threat of significant *Pgt* races including TTKSK, TRTTF, and TTTTF. Therefore, these varieties are not only highly valuable in current durum production, but they are also useful genetic resources for future durum breeding. To further diversify breeding germplasm, additional *Sr* genes for resistance to TTKSK, TRTTF, and TTTTF should be introduced from other sources. For example, *Sr13* provides a moderate level of resistance against TTKSK and resistance of varieties carrying *Sr13* could be enhanced if it were combined

with other effective *Sr* genes like *Sr2*, *Sr26*, *Sr39*, *Sr47*, *Sr56*, etc. (Singh et al. 2015). Stacking of *Sr* genes will play a significant role in development of future breeding lines with improved resistance against *Pgt* races, and this process could be expedited by using STARP or similar PCR-based SNP genotyping technologies.

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